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Remote ischemic conditioning to protect the heart in myocardial infarction: therapeutic intervention and underlying mechanisms

by

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To my family

From Karolinska Institutet, Department of Medicine,
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Stockholm, Sweden

Remote ischemic conditioning to protect the heart in myocardial infarction: therapeutic intervention and underlying mechanisms

AKADEMISK AVHANDLING

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ABSTRACT

Background

Urgent reperfusion of the occluded coronary artery halts the ischemic insult to the myocardium and is the single most important action to limit infarct size in ST-elevation myocardial infarction. However, reperfusion itself introduces an additional threat to the recovering ischemic myocardium whereby still viable cardiomyocytes suffer additional irreversible damage. This is referred to as reperfusion injury and significantly contributes to final infarct size. As of yet there is no established treatment to avoid or limit reperfusion injury, but promising results have been presented from early clinical trials of remote ischemic conditioning, whereby short cycles of non-harmful ischemia and reperfusion performed in remote tissue during the late stages of myocardial ischemia or early stages of myocardial reperfusion elicits an innate mechanism that ultimately terminates in cardioprotection. However, the exact underlying mechanisms are not fully known and additional clinical trials are needed to decide the possible role for remote ischemic conditioning in the treatment of patients with ST-elevation myocardial infarction. This thesis was undertaken to determine the effects of remote ischemic conditioning as an adjunct to primary percutaneous coronary intervention in patients with ST-elevation myocardial infarction and to explore underlying mechanisms.

Studies I and II

The effect of remote ischemic conditioning performed during the late stages of ischemia and at reperfusion as an adjunct to primary percutaneous intervention was investigated in 115 patients with anterior ST-elevation myocardial infarction. Infarct size as evaluated by cardiac magnetic resonance imaging during the first week and six months after the myocardial infarction, as well as clinical outcomes up to three years of follow-up were not affected by remote ischemic conditioning.

Study III

The involvement of the glucagon-like peptide-1 receptor in the signaling pathway of remote ischemic conditioning was explored in twelve healthy males. Intravenous infusion of the glucagon-like peptide-1 receptor antagonist exendin(9-39) abolished the protection against endothelial ischemia-reperfusion injury provided by remote ischemic conditioning, as evaluated by ultrasound based measurement of flow mediated dilatation in the brachial artery.

Study IV

The effect of chronic ticagrelor treatment on endothelial function was evaluated in twenty male patients with a previous acute coronary syndrome. Chronic ticagrelor treatment was not associated with protection against endothelial ischemia-reperfusion injury or better basal endothelial function compared to after its discontinuation, as evaluated by ultrasound based measurement of flow mediated dilatation in the brachial artery.

Conclusions

Remote ischemic conditioning performed during the late stages of ischemia and at reperfusion as an adjunct to primary percutaneous coronary intervention in patients with anterior ST-elevation myocardial infarction does not lead to smaller myocardial infarct size or improved clinical outcomes. Remote ischemic conditioning utilizes a glucagon-like peptide-1 receptor dependent pathway to protect against endothelial ischemia-reperfusion injury. Chronic ticagrelor treatment does not provide protection against endothelial ischemia-reperfusion injury or improved basal endothelial function compared to after its discontinuation.

LIST OF ORIGINAL PAPERS

This thesis is based on the following original studies which will be referred to by their Roman numerals.

I Verouhis D, Sörensson P, Gourine A, Henareh L, Persson J, Saleh N, Settergren M, Sundqvist M, Tornvall P, Witt N, Böhm F, Pernow J. Effect of remote ischemic conditioning on infarct size in patients with anterior ST-elevation myocardial infarction. *Am Heart J 2016;181:66-73.*

II Verouhis D, Sörensson P, Gourine A, Henareh L, Persson J, Saleh N, Settergren M, Sundqvist M, Tengbom J, Tornvall P, Witt N, Böhm F, Pernow J. Long term effect of remote ischemic conditioning on infarct size and clinical outcomes in patients with anterior ST-elevation myocardial infarction. *Manuscript.*

III Verouhis D, Saleh N, Settergren M, Sörensson P, Gourine A, Pernow J. Remote ischemic conditioning protects against endothelial ischemia-reperfusion injury via a glucagon-like peptide-1 receptor-mediated mechanism in humans. *Int J Cardiol 2019;274:40-44.*

IV Verouhis D, Ekström M, Settergren M, Sörensson M, Pernow J, Saleh N. Ticagrelor does not protect against endothelial ischemia-reperfusion injury in patients with coronary artery disease. *Manuscript.*

SAMMANFATTNING

Trots flera decennier med stora framsteg inom forskning kring och behandling av kronisk kranskärlssjukdom och akut hjärtinfarkt, fortsätter dessa att vara dominerande hälsoproblem globalt och medför död och lidande för drabbade patienter och stora kostnader för samhället. Fortsatta ansträngningar för att utvidga kunskapen kring och utveckla behandlingen av dessa tillstånd är av största betydelse.

Riskfaktorer såsom högt blodtryck, höga blodfetter, rökning, diabetes mellitus, fetma, fysisk inaktivitet och ärftlighet ökar belastningen på hjärtats kranskärl och accelererar åderförkalkningen. De kranskärlsplack som bildas kan orsaka mer eller mindre uttalade förträngningar av kranskärlen. Dessa plack kan drabbas av inflammation varvid skörhet och sprickbildningar kan uppstå. När innehållet i ett sådant sprucket plack kommer i kontakt med det cirkulerande blodet aktiveras blodproppsbildande mekanismer. Den blodpropp som då bildas i anslutning till det spruckna placket kan leda till tilltäppning av det drabbade kranskärlet varvid blodförsörjningen till en del av hjärtat upphör. Till följd av syrebristen (*ischemi*) som då uppstår dör hjärtmuskelceller gradvis i det drabbade området - hjärtinfarkt. Om patienten överlever det akuta skedet kvarstår en ökad risk för tidig död, nedsatt hjärtfunktion och lidande, vilken korrelerar med utbredningen av hjärtinfarkten. För att begränsa celldöden och förbättra prognosen för patienten är skyndsamt återställande av blodflödet (*reperfusion*) av avgörande betydelse. Med hjälp av kateterburen teknik (perkutan coronar intervention - PCI) kan den tilltäppande blodproppen mekaniskt avlägsnas och den bakomliggande förträngningen vidgas, varvid blodflödet återställs och den skadliga syrebristen i hjärtmuskelvävnaden upphör. En andel av hjärtmuskelcellerna kommer därvid att ha drabbats av oåterkallelig skada och dött, men om blodflödet har återställts tillräckligt skyndsamt kommer också en andel av hjärtmuskelcellerna att ha lidit mindre uttalad syrebrist och endast tillfälligt skadats men med potential att återta sin funktion. Dock leder det plötsliga återvändandet av blodflöde även till en paradoxal negativ reaktion (*reperfusionsskada*) i den känsliga del av hjärtmuskeln som håller på att återhämta sig, varvid en andel av dessa celler skadas ytterligare och dör vilket resulterar i att hjärtinfarktens totala storlek blir väsentligt större. Det finns ännu idag ingen etablerad behandling som riktar sig mot reperfusionsskada trots att många olika behandlingsprinciper har utforskats extensivt, men den terapeutiska potentialen är stor. Den senaste tiden har dock djurstudier, experimentella mekanistiska studier hos människor och tidiga kliniska studier av *remote ischemic conditioning (RIC)* hos patienter med akut hjärtinfarkt visat lovande resultat. RIC bygger på princepen att korta, icke skadliga, cykler med ischemi och reperfusion i annan vävnad än hjärtat aktiverar ett kroppseget försvar som leder till skydd mot reperfusionsskada. De exakta mekanismerna är inte fullständigt kända, men hållpunkter för överföring av den skyddande signalen via nervbanor såväl som via blodet har presenterats. Syftet med denna avhandling var att undersöka effekten av RIC hos patienter med akut hjärtinfarkt samt att utforska möjliga underliggande verkningsmekanismer för skydd mot reperfusionsskada.

Studie I och II undersökte effekten av RIC (utfört genom cykler av uppblåsning av en blodtrycksmanschett runt ett lår) i tillägg till standardbehandling med PCI hos patienter med akut hjärtinfarkt. Utfallet utvärderades dels med magnetkameraundersökningar (*cardiac magnetic resonance – CMR*) inom en vecka och sex månader efter hjärtinfarkten och dels med analys av vårdhändelser och överlevnad upp till tre år efter hjärtinfarkten. Behandling med RIC resulterade inte i mindre hjärtinfarkt eller bättre hjärtfunktion akut

eller sex månader efter hjärtinfarkten. Inte heller noterades bättre överlevnad eller mindre hjärt-kärlsjuklighet upp till tre år efter hjärtinfarkten.

Studie III undersökte betydelsen av glukagon-lik peptid-1 (*GLP-1*) för den skyddande effekten av RIC mot reperfusionsskada. En modell av tillfällig reperfusionsskada på blodkärl (*endotelial reperfusionsskada*) i underarmen användes och utvärderades med hjälp av ultraljudsundersökning av blodkärlsfunktionen (*flödesmedierad dilatation - FMD*). GLP-1 receptorhämmaren exendin(9-39) blockerade den skyddande effekten av RIC mot endotelial reperfusionsskada.

Studie IV undersökte om långtidsbehandling med det blodplättshämmande läkemedlet *ticagrelor* skyddar mot reperfusionsskada eller medför bättre blodkärlsfunktion (endotelfunktion) jämfört med efter behandlingens avslut. Ticagrelor ingår som standardbehandling under en begränsad tidsperiod efter akut hjärtinfarkt. Tidigare studier har föreslagit att preparatet förutom sin blodplättshämmande effekt kan ha ytterligare skyddande effekter på hjärta och blodkärl genom alternativa verkningsmekanismer. Patienter med tidigare hjärtinfarkt och pågående behandling med ticagrelor studerades i en underarmsmodell av endotelfunktion baserad på ultraljudsmätning av FMD. Långtidsbehandling med ticagrelor resulterade inte i skydd mot endotelial reperfusionsskada eller bättre endotelfunktion jämfört med efter behandlingens utsättande.

Resultaten i denna avhandling:

1. Stödjer inte hypotesen att RIC skyddar hjärtat mot reperfusionsskada vid akut hjärtinfarkt, till skillnad från vad tidigare studier har antytt. För att bättre förstå denna skillnad i utfall behövs stora studier fokuserade på robusta utfallsmått och fortsatt utforskande av underliggande verkningsmekanismer för RIC.

2. Illustrerar utmaningen i att överföra fynd från djurstudier och experimentella mekanistiska studier hos människa till kliniska studier hos patienter med akut hjärtinfarkt.

3. Visar att RIC skyddar mot endotelial reperfusionsskada genom en GLP-1 receptorberoende mekanism hos människa, vilket indikerar att GLP-1 är involverad i RIC-medierat skydd mot reperfusionsskada. Detta är en helt ny kunskap som förtjänar att studeras närmare och erbjuder nya infallsvinklar och möjligheter att förstå verkningsmekanismen bakom RIC.

4. Visar att långtidsbehandling med ticagrelor hos patienter med tidigare hjärtinfarkt inte skyddar mot endotelial reperfusionsskada eller förbättrar endotelfunktionen jämfört med efter dess utsättning.

ABBREVIATIONS AND ACRONYMS

AAR	area at risk
ACE-I	angiotensin converting enzyme-inhibitor
ACh	acetylcholine
ACS	acute coronary syndrome
ADP	adenosine diphosphate
AKI	acute kidney injury
Akt	protein kinase B
ANOVA	analysis of variance
APPROACH	Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease
ARB	angiotensin II receptor blocker
ATP	adenosine triphosphate
AUC	area under curve
BARI	Bypass Angioplasty Revascularization Investigation
BMI	body mass index
BSA	body surface area
CABG	coronary artery bypass grafting
cAMP	cyclic adenosine monophosphate
CGRP	calcitonin gene-related peptide
CI-AKI	contrast-induced acute kidney injury
CKMB	creatine kinase-myocardial band
CMR	cardiac magnetic resonance
CRP	C-reactive protein
DBP	diastolic blood pressure
ECG	electrocardiogram
EIVD	endothelium-independent vasodilatation
eNOS	endothelial nitric oxide synthase
ENT1	equilibrative nucleoside transporter 1
ERK	extracellular regulated kinase
ESA	endocardial surface area
ESC	European Society of Cardiology
FMD	flow-mediated dilatation
FU	follow-up
GFR	glomerular filtration rate
GLP-1	glucagon-like peptide-1
GP-IIb/IIIa	glycoprotein IIb/IIIa
GSK3β	glycogen synthase kinase 3β
HDL	high-density lipoprotein
HF	heart failure
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance

IR	ischemia-reperfusion
IS	infarct size
JAK	Janus kinase
LAD	left anterior descending artery
LDL	low-density lipoprotein
LGE	late gadolinium-enhancement
LV	left ventricle
LVEDV	left ventricular end-diastolic volume
LVEF	left ventricular ejection fraction
LVESV	left ventricular end-systolic volume
MACCE	major adverse cardiac and cerebrovascular events
MaR	myocardium at risk
MBG	myocardial blush grade
MI	myocardial infarction
mPTP	mitochondrial permeability transition pore
MSI	myocardial salvage index
MVO	microvascular obstruction
NO	nitric oxide
PCI	percutaneous coronary intervention
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PKG	protein kinase G
PLATO	Platelet Inhibition and Patient Outcomes
RCA	right coronary artery
RIC	remote ischemic conditioning
RISK	Reperfusion Injury Salvage Kinase
ROS	reactive oxygen species
SAFE	Survivor Activator Factor Enhancement
SBP	systolic blood pressure
SDF-1	stromal cell-derived factor-1
SEM	standard error of the mean
SPECT	single photon emission computed tomography
SR	sarcoplasmic reticulum
STEMI	ST-elevation myocardial infarction
TG	triglycerides
TIMI	Thrombolysis In Myocardial Infarction
TnI	troponin I
TnT	troponin T
TTE	transthoracic echocardiogram

INTRODUCTION

Sudden obstruction of a coronary artery caused by thrombotic occlusion at the site of a ruptured atherosclerotic plaque leads to ischemia and subsequent ST-elevation myocardial infarction (STEMI) **(Figure 1)**. Urgent restoration of coronary blood flow by primary percutaneous coronary intervention (PCI) is the cornerstone of modern STEMI treatment and limits the extent of the myocardial infarction[1]. However, reperfusion itself introduces an additional threat to the myocardium, whereby ischemic but still viable cardiomyocytes at the end of ischemia are further injured by the return of blood flow[2]. This is referred to as reperfusion injury and is sometimes divided into four types: 1) myocardial stunning – transient dysfunction of previously ischemic but not irreversibly injured myocardium; 2) no-reflow phenomenon – paradoxical absence of coronary reflow in the infarct-related artery despite technically successful intervention towards the culprit lesion; 3) reperfusion arrhythmias – disturbances of cardiac rhythm occurring after successful reperfusion of the occluded coronary artery; 4) lethal reperfusion injury – cardiomyocyte death occurring after successful reperfusion of ischemic myocardium. Lethal reperfusion injury may contribute to as much as 50 % of the final infarct size **(Figure 1)**[3].

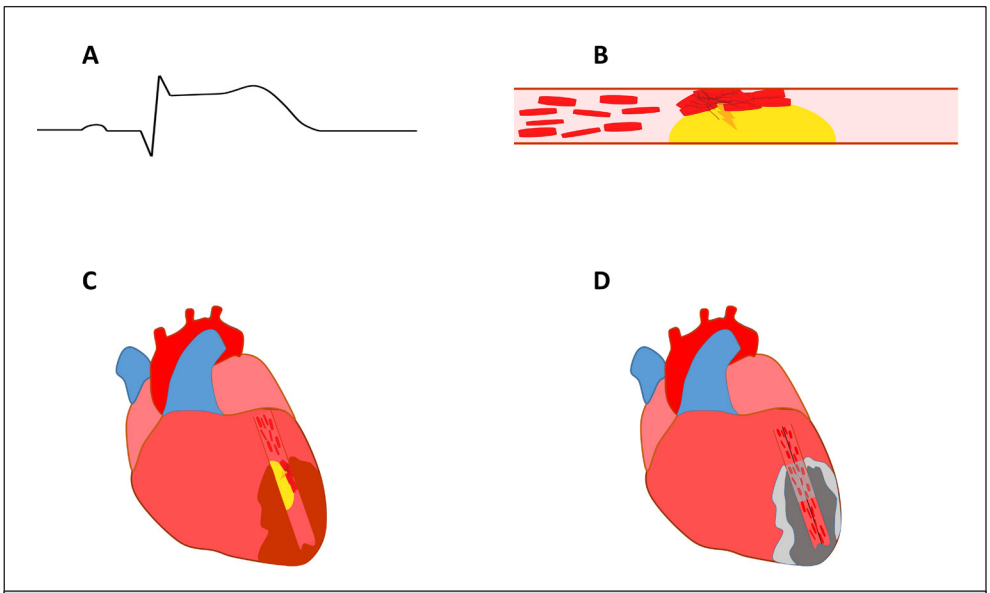

Figure 1. Ischemia-reperfusion injury in STEMI. A) ECG showing STEMI. B) STEMI is typically caused by acute thrombotic coronary occlusion at the site of a ruptured atherosclerotic plaque. C) Ischemia occurs in the myocardial area supplied by the occluded coronary vessel (dark red). D) Urgent reperfusion by primary PCI relieves ischemia and limits the extent of the myocardial infarction. Part of the ischemic myocardium has suffered irreversible ischemic injury and cardiomyocyte death (dark grey). A proportion of the ischemic myocardium is still alive at reperfusion (bright grey), but suffers additional damage from the return of blood flow – reperfusion injury - leading to enlargement of the infarcted myocardial area. ECG = electrocardiogram; PCI = percutaneous coronary intervention; STEMI = ST-elevation myocardial infarction.

In this thesis, the term ischemia-reperfusion injury will be used to refer to lethal reperfusion injury. There is still no established therapy to restrict reperfusion injury, but the large therapeutic potential is evident **(Figure 2)**.

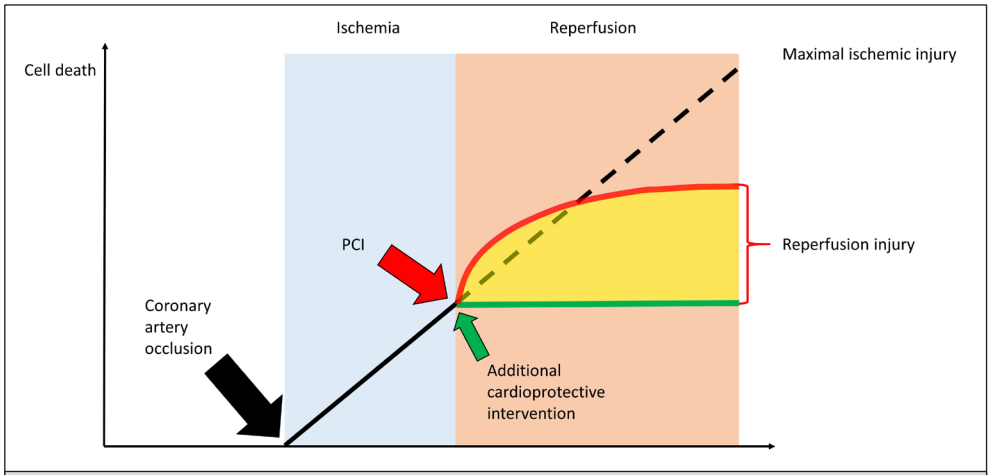

Figure 2. Ischemia-reperfusion injury. Shortly after coronary occlusion (black arrow) ischemic cardiomyocyte death ensues and the extent increases with time. Urgent reperfusion (red arrow), preferably with PCI limits ischemic cell death and the extent of myocardial infarction. However, although ischemia is relieved, cell death does not cease immediately at reperfusion as additional damage is conferred by reperfusion injury (yellow field), substantially contributing to final infarct size. A hypothetical cardioprotective approach directed towards reperfusion injury (green arrow) has the potential to significantly reduce final infarct size. Solid black line indicates progressing ischemic myocardial injury. Dashed black line indicates maximal extent of myocardial ischemic injury if no reperfusion is achieved. Red line indicates course of reperfusion injury. Green line indicates theoretical course in the event of effective intervention against reperfusion injury. PCI = percutaneous coronary intervention. (Adapted from Garcia-Dorado, Piper. Cardiovasc Res. 2006 Jan;69(1):1-3.)

Over the latest decades intense research has focused on finding ways to limit the extent of ischemia-reperfusion injury. While several different concepts have been explored, the technique of eliciting innate cardioprotective mechanisms through intermittent short cycles of ischemia and reperfusion in remote tissue - remote ischemic conditioning (RIC) has emerged as one of the most promising evolving strategies. However, clinical data are scarce and not entirely conclusive and the underlying mechanisms are far from fully understood.

Acute myocardial infarction

Prolonged strain from risk factors such as hypertension, hyperlipidemia, smoking, diabetes mellitus, obesity, physical inactivity and hereditary factors contributes to the formation of coronary atherosclerotic plaques and development of ischemic heart disease (IHD)[4-6]. IHD is a dominant health problem worldwide, leading to extensive morbidity, mortality and cost to society[7]. The Global Burden of Disease study estimated that IHD was prevalent in more than 110 million people and accounted for close to nine million deaths worldwide

in 2015[8]. In its most acute form, coronary artery disease culminates in rupture of the atherosclerotic coronary plaque leading to the formation of an occlusive coronary thrombus **(Figure 1)**[9-11]. The resulting transmural ischemia is typically manifested as acute chest pain and ST-segment elevation on the electrocardiogram (ECG). Extensive cardiomyocyte cell death spreads according to the wavefront phenomenon[12,13] and the risk of acute mortality is considerable. For patients surviving the acute phase, the risk of disabling chronic heart failure is substantial and correlates to myocardial infarct size[14]. Extensive research over the last decades has introduced several efficient cardioprotective strategies aimed at limiting the ischemic burden on the myocardium and counteracting the thrombotic activation[1]. Beta-blockers are beneficial as they decrease myocardial oxygen demand by reducing blood blood pressure, heart rate and myocardial contractility, while dual antiplatelet therapy with aspirin and a P2Y12 inhibitor (preferably ticagrelor) is the recommended mode of platelet inhibition. Importantly, the single most effective action to limit the final infarct size is to rapidly restore coronary blood flow. In some settings without access to acute cardiac catheterization, primary reperfusion is attempted with fibrinolytic compounds. However, coronary revascularization is most efficiently achieved through urgent primary PCI. PCI is typically performed by advancing a coronary guide catheter through the radial artery, to reach the ostium of the occluded coronary artery. Then, a coronary guidewire is passed through the coronary guide catheter, further on in to the coronary artery of interest and through the occlusion. Subsequently a coronary dilatation balloon is advanced over the coronary guidewire to the site of coronary occlusion, where it is inflated to remove the occlusion and dilate the underlying ruptured atherosclerotic plaque, thereby typically achieving restoration of coronary blood flow and instant relief of the ischemic threat to the myocardium. The procedure is typically concluded with the placing of a coronary stent over the ruptured coronary plaque in a similar manner. To maximize the gain of primary PCI it is important to avoid any time delay and guidelines set ≤90 minutes from first medical contact to reperfusion as a quality target[15].

Reperfusion injury

Since the first description of myocardial reperfusion injury in 1960 by Jennings et al.[16], extensive research has revealed a complex system of events initiated upon restoration of blood flow that may further hurt the ischemic heart. While the ultimate endpoint of reperfusion injury is cardiomyocyte death, several cell types are involved in reperfusion injury. Under physiological conditions as well as in the ischemia-reperfusion setting the cardiomyocytes are in deep interaction with the abundant endothelial cells lining the extensive vascular network surrounding the cardiomyocytes[17]. These endothelial cells in turn have an intense interplay with cells and molecules circulating in the vessels. While the exact mechanism of reperfusion injury still is partly unknown, several important steps have been identified. Opening of the mitochondrial permeability transition pore (mPTP) is a crucial event, leading to mitochondrial swelling and cell death **(Figure 3)**[18].

Reoxygenation

Upon reoxygenation of the ischemic (but still not irreversibly injured) myocardium an array of intracellular processes are initiated that terminate in hypercontracture of myofibrils, damage to the cell membrane and cell death. The cardiomyocyte accumulates Ca^{2+} and Na^{+} in the

cytosol during ischemia[19,20]. At reoxygenation, the Ca^{2+} pump of the sarcoplasmic reticulum (SR) is reactivated to clear Ca^{2+} from the cytosol into the SR[21,22]. As the large amounts of Ca^{2+} exceed the storage capacity of the SR, a continuous oscillating state of intake and release of Ca^{2+} is reached, which in turn induces malicious hypercontracture of the cardiomyocytes as well as opening of mPTPs[23]. The latter is considered a key event, leading to collapse of the inner mitochondrial membrane potential, uncoupling of oxidative phosphorylation, energy depletion, mitochondrial swelling and cell death[18]. Furthermore, Ca^{2+} overload activates calpains which degrade the cytoskeleton and damage the Na^+ pump of the cell membrane, further contributing to cell injury[24]. This additionally contributes to Ca^{2+} overload as an intact function of the Na^+ pump of the cell membrane is important to uphold a proper Na^+ gradient to the extracellular compartment, which is required by the Na^+/Ca^{2+} exchanger of the cell membrane in order to relieve the excess Ca^{2+} [22]. Moreover, the cytoskeleton of the recently ischemic cardiomyocyte is hypersensitive to the forceful contractions of the myofibrils due to Ca^{2+} overload and upon re-energization the cell is prone to go into a deleterious state of irreversible hypercontracture[25].

In addition, upon reoxygenation there is a massive increase of reactive oxygen species (ROS) which are formed in cardiomyocytes, endothelial cells and infiltrating neutrophils[26]. The large amounts of ROS that are generated widely exceed the capacity of endogenous scavenging systems. ROS exert several detrimental effects via the reduction of bioavailability of nitric oxide (NO), opening of mPTP and further attraction of neutrophils and activation of platelets and complement that all contribute to myocardial injury[27,28].

Restoration of pH

Reperfusion of the occluded artery leads to rapid correction of the acidic extracellular milieu, leading to a massive H^+ gradient over the cardiomyocyte membrane. Subsequently the Na^+/H^+ exchanger and the Na^+/HCO_3^- co-transporter are activated to quickly correct this

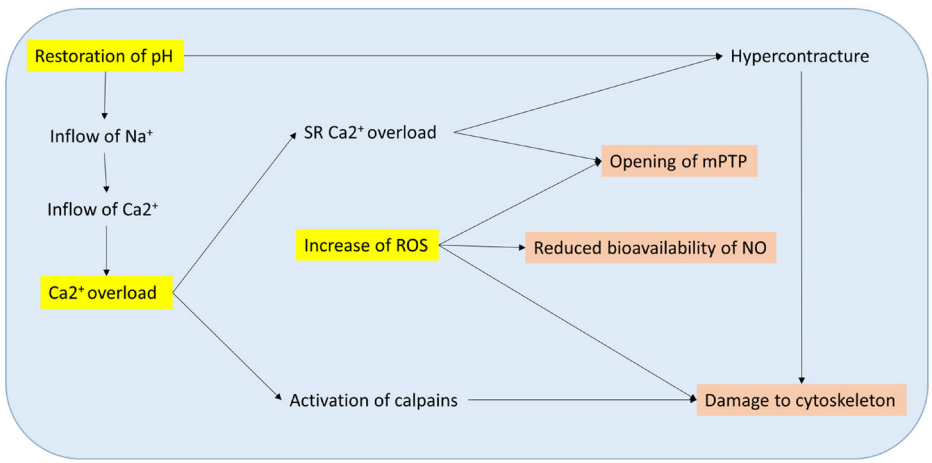

Figure 3. Simplified scheme of key mechanisms involved in the cardiomyocyte in reperfusion injury. NO = nitric oxide; mPTP = mitochondrial permeability transition pore; ROS = reactive oxygen species ; SR = sarcoplasmic reticulum.

imbalance[29]. However, these events have negative consequences as the sudden restoration of pH leads to withdrawal of the inhibitory effect of acidosis on the hypercontraction-prone myofibrils. In addition, the inflow of Na^+ may exceed the capacity of the Na^+ pump in the cell membrane to maintain an adequate Na^+ gradient for the Na^+/Ca^{2+} exchanger to function properly, leading to reversal of its mode with resulting Ca^{2+} inflow to the cardiomyocyte, further exacerbating the negative consequences of Ca^{2+} overload.

Restoration of tissue osmolality

Reperfusion of the occluded artery also causes rapid correction of the hyperosmolar extracellular environment, leaving a substantial gradient to the still hyperosmolar cardiomyocyte due to accumulation of Na^+ and anaerobic metabolites[30]. This leads to rapid inflow of water and swelling of the fragile hypercontracting postischemic cell which causes further cell damage and disruption of the cell membrane.

Remote ischemic conditioning

The concept of *ischemic conditioning* whereby innate organ protective properties are activated by short non-harmful cycles of ischemia and reperfusion before, during or shortly after the deleterious long period of ischemia and subsequent reperfusion has been extensively explored since the first demonstration of ischemic conditioning by Murry et al. three decades ago[31]. They showed that brief intermittent occlusions of the left anterior descending artery (LAD) prior to a prolonged infarct-generating occlusion and subsequent reperfusion of the same vessel was cardioprotective in dogs. While the efficacy of ischemic *pre*-conditioning has been consistent over a large number of species[32], this approach is applicable only in situations where ischemia and reperfusion is planned, and hence not an alternative in the STEMI-setting in which the ischemic insult cannot be foreseen. However, it is also possible to achieve similar protection by performing the conditioning stimulus immediately after the deleterious ischemic insult and during the initial phase of reperfusion[3]. This approach is more compatible with the STEMI-setting, where such ischemic *post*-conditioning can be performed locally in the infarct related artery by short re-occlusions of the vessel during PCI. Several clinical studies of such *local* ischemic post-conditioning in patients with STEMI have been performed, but results have been inconclusive[33]. Another way to induce ischemic conditioning is by performing the conditioning stimulus in tissue distant to the area at risk – *remote* ischemic conditioning (RIC). This was initially demonstrated by Przyklenk et al. who performed the conditioning stimulus in the circumflex artery in dogs to limit the extension of the infarcted area following occlusion of the LAD[34]. In fact, the conditioning stimulus can even be performed in a remote organ, as originally described by Gho et al.[35]. It has been demonstrated in numerous animal studies that brief repetitive cyles of ischemia and reperfusion in remote tissue such as in the limbs, kidneys, intestines, skin and brain induce protection in the heart. Furthermore, the RIC-stimulus can be performed before (remote ischemic *pre*conditioning), during (remote ischemic *per*conditioning) or after (remote ischemic *post*conditioning of the deleterious ischemic period in a manner similar to that demonstrated in local conditioning procedures[36]. Mechanistic studies in humans using surrogate markers have shown that this mechanism exists in humans[37] and early studies of RIC as an adjunct to PCI in patients with STEMI have shown promising results which are described in detail below[38-48]. The exact mechanism by which RIC leads to end

organ protection is far from fully understood but there is evidence for humoral[49] as well as neural involvement[49-54]. The general idea is that RIC initiates a remote signal (neural or/and humoral) that is transmitted (neurally or/and humorally) to the end organ where it induces protection.

RIC-mechanisms

While the exact way by which RIC leads to end-organ protection still is unknown, a common approach is to separate the mechanism into (1) initiation of a conditioning stimulus by RIC in a remote organ, (2) transfer of the conditioning signal to the end-organ and (3) exertion of end-organ protection **(Figure 4)**.

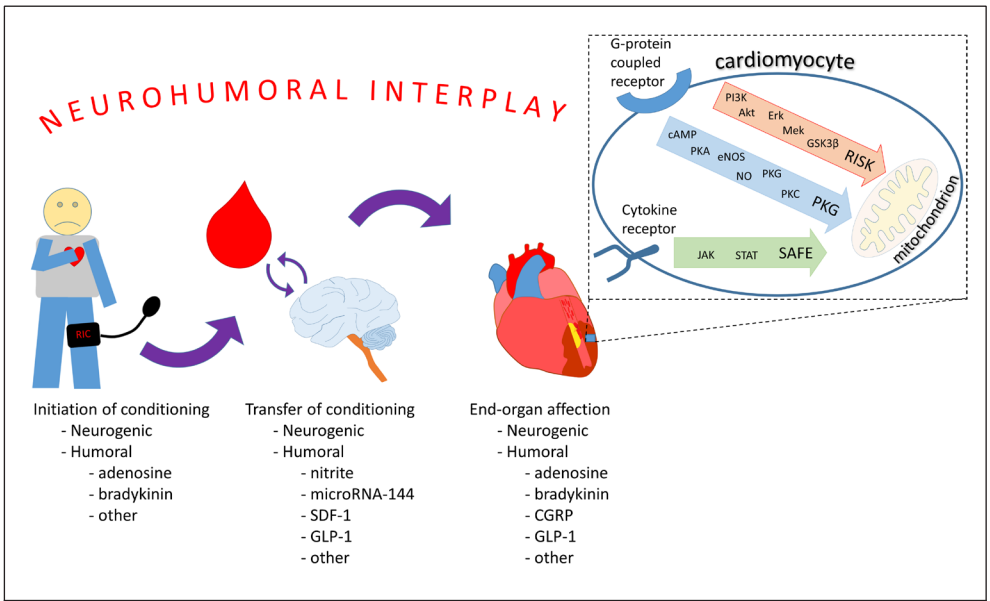

Figure 4. Simplified schematic overview of proposed transmission of the RIC signal. Cycles of ischemia and reperfusion in remote tissue initiate a conditioning signal that is transmitted through a neurohumoral interplay to reach the heart. There it activates cardioprotection, predominatly through inhibition of the mitochondrial permeability transition pore. Proposed contributors to signaling are presented, but the precise mechanism is not fully known. Red arrow denotes the RISK (Reperfusion Injury Salvage Kinase) pathway. Blue arrow denotes the PKG (protein kinase G) pathway. Green arrow denotes the SAFE (Survivor Activator Factor Enhancement) pathway. Akt = protein kinase B; cAMP = cyclic adenosine monophosphate; CGRP = calcitonin gene-related peptide; eNOS endothelial nitric oxide synthase; ERK = extracellular regulated kinase; GLP-1 = glucagon-like peptide-1; GSK3β = glycogen synthase kinase 3β; JAK = Janus kinase; NO = nitric oxide; PI3K = phosphatidylinositol (4,5)-bisphosphate 3-kinase; PKA = protein kinase A; PKC = protein kinase C; PKG = protein kinase; RIC = remote ischemic conditioning; SDF-1 = stromal cell-derived factor-1; STAT = signal transducer and activator of transcription. Adapted from Heusch. Circ Res. 2015;116:674-699 and Hausenloy, Yellon. Nat Rev Cardiol. 2016 Apr;13(4):193-209.

(1) Initiation of a conditioning stimulus by RIC

The nature of the conditioning stimulus that is created by short cycles of ischemia and reperfusion is still unknown. However, there is strong evidence that this stimulus activates nerve fibers within the conditioned tissue, to relay the signal further, as demonstrated by the findings that sectioning of sciatic and femoral nerves abolishes cardioprotection induced by RIC in the limb[55-57]. While several endogenous compounds have been suggested to be released in peripheral tissue in response to RIC, considerable attention has been focused on adenosine[58,59] and bradykinin[60] acting as autacoids, either activating afferent nerve fibers to further relay the conditioning signal or stimulating the release of other humoral factors.

(2) Transfer of the conditioning signal to the end-organ

The involvement of a blood-borne factor carrying the conditioning signal is strongly supported by the fact that plasma dialysate from a conditioned individual contains a conditioning stimulus that can be successfully transferred to another individual to provide cardioprotection[61-63]. This can even be done across species[64]. In addition, a transplanted denervated heart can be protected from ischemia-reperfusion injury by RIC[65]. Extensive efforts have been made to identify a humoral factor involved in transferring the conditioning signal. This factor is suggested to be of lower molecular weight than 15 kDa[61] and there is support for the involvement of several possible factors, such as nitrite[66], stromal cell-derived factor-1[67] and microRNA-144[68]. Thus, while the precise nature of this humoral transfer still is not known, it is likely that the mechanism is complex and involves several steps including an intricate interplay with the neural system. This is supported by the finding that the possibility to transfer cardioprotection through plasma dialysate from a conditioned individual is lost if the donor suffers from diabetic neuropathy[49] or has been vagotomized[53]. In addition, cardioprotection by RIC can be blocked by the autonomic ganglion blockers hexamethonium[35,53] and trimetaphan[37] and by the muscarinic receptor blocker atropine[50,52,53]. Further evidence for the critical involvement of the neural system is that sectioning of the spinal cord or the vagal nerve abolishes cardioprotection provided by RIC[50,57]. Indeed, there is accumulating evidence for the crucial involvement of the vagal nerve and recent data show that selective sectioning of the posterior gastric branch of the vagal nerve inhibits cardioprotection by RIC, while electrical stimulation of the same branch affords cardioprotection[69]. As the posterior gastric branch of the vagal nerve modulates release of glucagon-like peptide-1 (GLP-1)[70,71], this merits further interest as it links RIC to the established cardioprotective properties of GLP-1[72-76].

While there is evidence for a combined neurohumoral pathway transferring the cardioprotective signal, the exact mode of interaction between the neural and humoral components as well as their communication with the end-organ remains elusive. The heart receives abundant innervation which could be responsible for relaying the cardioprotective signal to the cardiomyocytes[77]. This view has been challenged by the fact that RIC induces cardioprotection in isolated heart models such as Langendorff-perfused preparations[61]. However, these hearts retain their extensive intrinsic cardiac innervation including local neural loops which regulate cardiac function[78]. The involvement of the intrinsic cardiac innervation in RIC is indicated by the finding that the cardioprotective effect on an isolated heart of dialysate from a conditioned individual is lost in the presence of the ganglion blockers hexamethonium and atropine[53]. It has been suggested that a humoral factor

may activate these intrinsic cardiac circuits to induce end-organ protection[53]. However, the indispensability of intrinsic neural involvement has been questioned by the finding that isolated cardiomyocytes are protected from ischemia-reperfusion injury by dialysate from conditioned rabbits in an opioid receptor-dependent manner[61,79]. Furthermore, an interaction with adenosine receptors seems to be present[79]. Whether acting via the intrinsic cardiac innervation or not, additional studies support adenosine[58,80,81] and opioids[82,83] as possible end-organ effectors. In addition, several other ligands have been explored[84], of which bradykinin[60,85] and calcitonin gene related peptide[86] have received the most attention.

(3) Exertion of end-organ protection
Final exertion of cardioprotection in RIC is a complex, multifactorial and not fully understood matter. The general view is that the conditioning signal reaches the cardiomyocyte and activates intracellular protective kinases that exert protection mainly through effects on the mitochondria, primarily by inhibiting the deleterious opening of the mPTP which is central to reperfusion injury **(Figure 4)**[84,87]. In addition, there is evidence for involvement of the endothelium[37], anti-inflammatory effects[88] and decreased thrombotic activity[28] in exerting the full cardioprotective effect of RIC.

RIC in STEMI
The simple application of RIC using a blood pressure cuff inflated above systolic blood pressure (SBP) has made it feasible to implement into clinical studies. Over the last few years several clinical studies investigating the efficacy of RIC have been performed which indicate that RIC may have a place as an adjunct to primary PCI in STEMI **(Table I)**.

The first study of the effect of RIC in patients with STEMI was presented by Bøtker et al. in 2010[38]. Four cycles of RIC of the upper limb before PCI was associated with a larger myocardial salvage index (MSI) compared to standard therapy as measured by single photon emission computed tomography (SPECT). In addition, a follow-up study showed that major adverse cardiac and cerebrovascular events (MACCE) were reduced[89]. Rentoukas et al. found that ST-resolution was improved and peak troponin I levels were decreased by three cycles of RIC of the upper limb during late ischemia and early reperfusion in patients with STEMI[39]. In addition, the effect was more pronounced by simultaneous infusion of morphine. Similar findings were presented by Crimi et al.[40]. Three cycles of RIC of the lower limb initiated at coronary reperfusion improved ST-resolution and lowered levels of creatine kinase-myocardial band (CKMB) in patients with STEMI. However, in a subgroup of patients undergoing cardiac magnetic resonance (CMR), infarct size was unchanged. White et al. used CMR to measure myocardial infarct size as primary outcome in a trial evaluating the effect of four cycles of RIC of the upper limb prior to primary PCI in patients with STEMI[41]. Their main finding was that absolute infarct size was reduced. However, also myocardial edema determined by CMR, which is considered to reflect MaR, was smaller in the intervention group. Thus CMR based MSI was not significantly different between study groups, which somewhat complicates the interpretation. However, “hybrid MSI” based on infarct size from CMR and MaR from angiographic scores was improved in the RIC group. Three cycles of lower limb RIC starting at reperfusion with primary PCI did not affect the primary outcome LVEF or left ventricular remodeling at 6 months as assessed by echocardiography in a trial by Elbadawi et al.[46]. Likewise, peak CKMB was neutral between study groups, but a better ST-resolution was noted in the RIC group. Liu et al. utilized CMR to show less microvascular

Table I. Summary of trials evaluating RIC as an adjunct to primary PCI in STEMI. AAR = area at risk; AUC = area under curve; AKI = acute kidney injury; CI-AKI = contrast induced acute kidney injury; CKMB = creatine kinase-myocardial band; CMR = cardiac magnetic resonance; GFR = glomerular filtration rate; HF = heart failure; IS = infarct size; LV = left ventricle; LVEF = left ventricular ejection fraction; MaR = myocardium at risk; MBG = myocardial blush grade; MI = myocardial infarction; MSI = myocardial salvage index; MVO = microvascular obstruction; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning; SPECT = single photon emission computed tomography; TnI = troponin I; TnT = troponin T; TTE = transthoracic echocardiogram.

Trial	Setting/Design	Patients	RIC-design	Primary outcome	Additional variables	Comments
Bøtker et al.[38] (2010) **Sloth et al.**[89] **Munk et al.**[161]	Randomized. Single center. No previous MI.	142 for primary outcome analysis. (251 for intention to treat analysis of secondary outcomes.)	Perconditioning. 4 upper arm RIC cycles initiated during ambulance transport.	Better MSI at 30 days (SPECT).	Reduced MACCE in RIC group. Better LVEF at 24 h, but not after 30 days. No effect on absolute IS (SPECT), peak TnT, ST-resolution and TIMI-flow after PCI. Reduced incidence of major adverse cardiac events (driven by a lower rate of HF) at 3.6 years follow-up.	Subgroup analyses of cases with large MaR found more pronounced effect on MSI, better left ventricular function acutely and persisting at 30 days for the RIC group.
Rentoukas et al.[39] (2010)	Randomized. Single center. The additive effect of iv morphine was examined in a third group.	96.	Per-postconditioning. 3 upper arm RIC cycles initiated approximately 10 minutes before primary PCI.	Higher proportion of patients achieving full ST-resolution.	Lower peak TnI.	Adding morphine to RIC resulted in higher degree of ST-resolution.
Crimi et al.[40] (2013)	Randomized. Single center. First anterior STEMI.	96 for primary outcome analysis.	Postconditioning. 3 lower limb RIC cycles initiated at reperfusion.	Lower AUC CKMB.	Better ST-resolution. No effect on absolute or relative IS at acute or follow-up CMR (at 4 months). Smaller extent of myocardial edema on CMR in the RIC group. No effect on AKI overall. For patients with low preprocedural GFR, RIC was associated with lower postprocedural creatinine.	Subgroup of 77 patients underwent CMR for secondary outcome analyses. T2-weighted CMR was used for evaluation of myocardial edema.
Prunier et al.[42] (2014)	Randomized. Multicenter. LAD or RCA occlusions only. The additive effect of local postconditioning was examined in a third group.	55 for primary outcome analysis.	Perconditioning. 3 upper arm RIC cycles initiated at arrival to catheterization laboratory.	Reduced AUC CKMB not reaching statistical significance.	Lower AUC CKMB/AAR (assessed by LV angiography and Field method). Lower peak CKMB and peak CKMB/AAR.	Adding local postconditioning to RIC did not affect outcomes.
White et al.[41] (2015)	Randomized. Single center. Anterior STEMI. Occluded culprit artery. No previous MI.	83 for primary outcome analysis. (197 for per protocol analysis of secondary outcomes.)	Per-postconditioning. 4 upper arm RIC cycles initiated at arrival to hospital.	Smaller absolute IS on day 3-6 (CMR).	Lower 24 h TnT. Smaller extent of myocardial edema on CMR in the RIC group. No effect on MSI by CMR. Better MSI by "hybrid method" (IS from CMR combined with MaR from BARI or APPROACH).	Myocardial edema was evaluated by T2-mapping CMR.

Trial	Setting/Design	Patients	RIC-design	Primary outcome	Additional variables	Comments
Eitel et al.[43] (2015) **Stiermaier et al.**[90]	Randomized. Single center. The effect of RIC combined with local postconditioning was studied but not RIC alone.	491 for primary outcome analysis. (692 for intention to treat analysis of secondary outcomes.)	Perconditioning. 3 upper arm RIC cycles initiated at arrival to hospital.	Better MSI on day 2-5 (CMR).	Better ST-resolution. No effect on peak CKMB, absolute IS, MVO, LV size or function. No effect on clinical outcomes at 6 months follow-up. Reduced incidence of major adverse cardiac events (driven by a lower rate of HF) at 3.6 years follow-up.	RIC-protocol allowed to be finished as postconditioning if not enough time to finish before PCI.
Yamanaka et al.[44] (2015)	Randomized. Multicenter.	94.	Perconditioning. 3 upper arm RIC cycles initiated at arrival to hospital.	Lower incidence of CI-AKI.	Lower peak CKMB. Lower incidence of ventricular arrhythmias. Combined clinical endpoints not significantly altered.	CI-AKI was defined as an increase in serum creatinine of more than 0.5 mg/dL or 25% of the baseline value 48–72 h after injection of contrast medium.
Verouhis et al. (2016)	Randomized. Multicenter. Anterior STEMI. No previous MI.	93 for primary outcome analysis. (115 for analysis of secondary outcomes.)	Perpostconditioning. RIC of lower limb to be initiated at arrival to catheterization laboratory. A least one cycle before PCI and 4 thereafter.	No effect on MSI on day 4-7 (CMR).	No effect on absolute IS, MVO, LV size, function or recovery on acute or follow-up CMR (after 6 months). No effect on clinical outcomes up to three years of follow-up.	MaR was evaluated using early contrast-enhanced steady state free precession images.
Liu et al.[45] (2016)	Randomized. Single center. No previous MI.	119.	Perconditioning. 4 upper arm RIC cycles initiated during ambulance transport.	Less MVO on day 3-7 (CMR).	Smaller absolute IS and better LV function on CMR day 3-7. Lower peak CMKB and better MBG. No effect on ST-resolution or TIMI-flow after PCI. No effect on clinical outcomes up to one year.	MaR was not evaluated.
Elbadawi et al.[46] (2017)	Randomized. Single center. Anterior STEMI.	60.	Postconditioning. 3 lower limb RIC cycles initiated at reperfusion.	No difference in LVEF (nor at index hospitalization neither after 6 months) or LV remodeling (after 6 months) as evaluated by TTE.	Better ST-resolution. No effect on peak CKMB, TIMI-flow, MBG or AKI. No effect on clinical outcomes at 6 months.	
Cao et al.[47] (2018)	Randomized. Single center. No previous MI.	80.	Postconditioning. 4 upper arm cycles at reperfusion.	CKMB at 0.5, 8 and 72 hours lower, but not at 24 and 48 hours. AUC CKMB lower. Better LVEF after 7 days. Less AKI. No effect on TIMI-flow.		Not clear what were primary and secondary endpoints.
Gaspar et al.[48] (2018)	Randomized. Single center.	448.	Per-postconditioning. 3 upper arm cycles initiated approximately 10 minutes before PCI.	Lower 2 year incidence of cardiac mortality and hospitalization for HF.	No effect on TIMI-flow, AKI or peak or AUC TnI. Reduced incidence of acute HF and need for inotropes. Better recovery of poor LV function.	

obstruction, smaller absolute infarct size and better acute LVEF in response to four cycles of RIC of the upper arm during ambulance transport to primary PCI[45]. Additionally, peak CKMB was lower in the RIC group and myocardial blush grade was higher. However, no effect on ST-resolution was noted. Lower peak CKMB and 72 h area under curve CKMB together with less acute kidney injury and better LVEF after seven days was reported from Cao et al. in response to four cycles of RIC of the upper arm, starting within one minute of reperfusion by primary PCI[47]. Similarly, Yamanaka et al. found lower peak CKMB and less acute kidney injury together with less tendency for ventricular arrhythmias in a group of patients receiving three cycles of RIC of the upper arm before primary PCI[44]. However, LVEF assessed by echocardiography two weeks after the STEMI was not affected. Using a protocol of three cycles of RIC of the lower limb during late ischemia and early reperfusion in 448 patients with STEMI undergoing primary PCI, Gaspar et al. found that patients in the RIC group had lower incidence of the combined primary endpoint of cardiac mortality and hospitalization for heart failure as well as for the individual components[48]. Additionally, the incidence of acute heart failure was lower in the RIC group and patients with more severe heart failure had better recovery of left ventricular function at one year follow-up.

It has also been hypothesized that the combination of different interventional strategies such as remote and local conditioning may have additive effects. Prunier et al. found that three cycles of upper limb RIC before PCI reduced CKMB levels but adding local postconditioning to the protocol had no effect[42]. Eitel et al. showed that three cycles of upper limb RIC prior to PCI combined with local postconditioning in the infarct related artery in patients with STEMI resulted in a greater MSI when compared to standard therapy as measured with CMR[43]. Additionally, a reduced incidence of major adverse cardiac events (driven by lower rate of heart failure) over a median of 3.6 years follow-up was demonstrated[90]. However, the effect of RIC alone was not studied in this trial.

Adding RIC to thrombolysis has also been investigated. Four cycles of upper arm RIC initiated before reperfusion with streptokinase resulted in lower 24 h area under curve CKMB and 24 h area under curve troponin T[91].

Thus, RIC in adjunction to primary PCI in STEMI has shown promising results in early studies, but as presented in **Table II**, the picture is not entirely conclusive. While several of the studies have indicated a positive effect of RIC using absolute measures of infarct size as primary endpoints, markers of infarct size taking into consideration MaR have been more difficult to interpret. Furthermore, all studies so far have been too limited in sample size for proper evaluation of hard clinical outcomes. Nonetheless, these early data indicate that RIC may have cardioprotective properties in patients with STEMI. Considering the huge potential clinical benefit if proven to protect against reperfusion injury in patients with STEMI, further studies based on robust measures of infarct size and clinical outcomes in large sample studies are warranted. Of additional importance, the most solid support for the effect of RIC in STEMI comes from the trial by Bøtker et al., in which RIC was initiated in the ambulance and completed before reperfusion therapy[38]. However, in several settings the prerequisites for such a setup may be lacking (for instance very short ambulance transport, less coordinated prehospital organization or patients arriving to the hospital by other means of transport than ambulance). In those cases, a RIC-protocol needs to be initiated at arrival in the catheterization laboratory but completed during PCI in a per-post design including the final conditioning cycles after reperfusion to avoid delaying reperfusion.

Myocardium at risk

The use of absolute infarct size as endpoint in clinical studies of cardioprotection against ischemia-reperfusion injury has the limitation that it is sensitive to several factors, some of which are difficult to control for (e.g. localization of coronary occlusion, temporal variation of degree of coronary occlusion, extent of collaterals and TIMI-flow). The consequence of this is a substantial variability in absolute infarct size within the study groups and thus need for large sample sizes in trials based on absolute infarct size as primary outcome measure. To overcome this, infarct size may be evaluated in relation to the MaR in each subject[92]. MaR refers to the ischemic myocardial area at risk for irreversible injury from ischemia-reperfusion. Coronary angiography provides the possibility to evaluate MaR using models based on the location of the culprit lesion and distribution of the coronary arteries. Several such models have been developed and provide prognostic information[93,94]. However, these scores are anatomically based assumptions of extent of ischemia rather than measurements of actual ischemia in each subject. The gold standard for evaluation of MaR in clinical studies of myocardial ischemia-reperfusion injury is SPECT[95-97], which is based on the lack of uptake and accumulation of isotope in non-perfused myocardial areas during coronary occlusion. However, SPECT requires injection of the isotope prior to coronary reperfusion and subsequent imaging within a few hours, which impairs its usefulness under certain circumstances. Additionally it exposes the patient to radiation. More recently, CMR-based techniques to evaluate MaR have gained increased popularity as they do not risk impairing the acute management of patients and offer a more convenient time window for performing the examination. CMR provides the possibility to determine MaR and infarct size in a single examination within a week from the myocardial infarction. Evaluation of MaR based on the endocardial extent of infarction (endocardial surface area (ESA) method))[94] has been used in studies of cardioprotection[98]. However, this method may underestimate MaR especially in cases with successful cardioprotective intervention[99]. Most utilized methods to evaluate MaR by CMR are essentially based on detection of myocardial edema. In the ischemia-reperfusion setting several mechanisms contribute to the formation of edema[100]. The major constituent of the myocardium is water, most of it being intracellular and predominantly bound to proteins and other molecules. Within a few minutes of ischemia, edema of the cardiomyocyte occurs due to failure of the Na^+/K^+ pump with subsequent accumulation of intracellular Na^+ and increased osmotic pressure leading to increase of intracellular water content. Furthermore, ischemia-induced acidosis leads to conformational changes of water-binding molecules, resulting in release of bound water. Additionally, prolonged ischemia causes damage to capillary membranes, resulting in leakage of water from the intravascular to the interstitial space. Moreover, reperfusion of the ischemic myocardium substantially contributes to myocardial edema. Reperfusion leads to rapid restoration of extracellular osmotic pressure, increase of extracellular water content and subsequently through osmotic pressure also increase of intracellular edema. The degree of water content in the myocardium is reflected in CMR-imaging and additionally the distinct properties of free vs bound water are manifested. Thus, the extent of myocardial edema as evaluated by CMR is a convenient surrogate marker for MaR. T1- and T2-weighted imaging reliably depict MaR up to one week after the myocardial infarction[101,102]. Contrast-enhanced steady state free precession imaging provides superior image quality to T2-weighted imaging and has been validated against SPECT[103] and angiographic scores[104]. Still, there has been some debate regarding the validity of CMR based evaluation of MaR[105,106] and the most appropriate timing

Table II. Variables in studies of RIC as an adjunct to primary PCI in STEMI. MaR on CMR is edema-based. MSI on CMR is derived from IS and edema-based MaR. Hybrid MSI is derived from IS and angiographically determined MaR. Green box indicates a positive effect of RIC. Blue box indicates a neutral effect of RIC. Yellow box indicates somewhat complex interpretation of the variable. Grey box indicates that the variable was not evaluated or clearly reported. # indicates the primary endpoint of the trial.

Variables			**Bøtker**[38]	**Rentoukas**[39]	**Crimi**[40]	**Prunier**[42]	**White**[41]
Cardiac enzymes	TnT	Peak or point					
		AUC					
	TnI	Peak or point					
		AUC					
	CKMB	Peak or point					
		AUC			#	#	
ST-resolution				#			
CMR	IS						#
	FU IS						
	MSI						
	FU MSI						
	Hybrid MSI						
	MaR						
	MVO						
SPECT	IS						
	MSI		#				
	MaR						
LV parameters from CMR or TTE	LVEF						
	LV size						
	LV recovery at FU						
Renal function							
Angiographic	TIMI-flow						
	MBG						
Clinical	Early combined						
	Late combined						
	Early HF						
	Late HF						
Comments			Subgroup analysis of cases with large MaR showed more pronounced effect on MSI, better LV function acutely and persisting at 30 days for the RIC group.		Smaller extent of myocardial edema in the RIC group. No effect on renal function overall but for patients with low pre-procedural GFR, RIC was associated with lower postprocedural creatinine.	CKMB AUC was not significantly reduced. CKMB AUC/AAR however was. Adding local postconditioning to the protocol did not alter outcomes.	Smaller extent of myocardial edema in the RIC group. TnT was lower at 24 h but not at other time points.

AUC = area under curve; CKMB = creatine kinase-myocardial band; CMR = cardiac magnetic resonance; FU = follow-up; GFR = glomerular filtration rate; HF = heart failure; IS = infarct size; LV = left ventricle; LVEF = left ventricular ejection fraction; MaR = myocardium at risk; MBG = myocardial blush grade; MSI = myocardial salvage index; MVO = microvascular obstruction; SPECT = single photon emission computed tomography TnI = troponin I; TnT = troponin T; TTE = transthoracic echocardiogram.

Yamanaka[44]	**Verouhis**	**Liu**[45]	**Elbadawi**[46]	**Cao**[47]	**Gaspar**[48]
	#				
		#			
			#		
			#		
#					
					#
	MaR was larger in the RIC group.			Not clear what were primary and secondary outcomes.	

for it, considering the bimodal pattern of edema after myocardial infarction[107-110]. However, a recent study in pigs validated contrast-enhanced steady state free precession imaging and T2-weighted imaging against histopathology to measure MaR seven to ten days after LAD artery occlusion and found good agreement, confirming its applicability in this time frame[111].

The endothelium

The human arterial wall is a three-layered structure. The media is separated from the outer adventitia and the inner intima by the external and internal elastic membranes and consists mainly of a layer of smooth muscle cells surrounded by extracellular matrix. The external adventitia is a collagen-rich connective tissue embedding the nervous (nervi vasorum) and vascular (vasa vasorum) supply of the artery. The intima consists of a single layer of endothelial cells, the endothelium, supported by loose connective tissue.

The endothelium is a highly active physiological entity with several important features. It is involved in the regulation of vascular tone and permeability, vessel wall inflammatory mediators and hemostasis[112]. The endothelium exerts its physiological properties mainly through endocrine and paracrine mediators. NO is the most extensively investigated endothelium derived factor[113]. It is formed from the amino acid L-arginine under the action of endothelial nitric oxide synthase (eNOS) and mediates several cardinal functions of the endothelium. However, endothelial function is not consistent. Aging as well as common conditions such as atherosclerosis, diabetes mellitus, hypertension, hyperlipidemia, obesity and smoking lead to impaired endothelial function related to reduced bioavailability of NO, owing to its reduced synthesis and increased degradation[114]. Furthermore, endothelial dysfunction provides prognostic information as it is associated with progression of atherosclerosis and predicts future cardiovascular clinical events[115]. Interestingly, endothelial dysfunction is an important event in development of myocardial ischemia-reperfusion injury during myocardial infarction by contributing to inflammation and oxidative stress[116,117].

Assessment of endothelial function was originally derived from the response to intracoronary infusion of acetylcholine (Ach)[118]. Under normal conditions, coronary arteries respond to this stimulus by dilatation due to release of NO. In patients with coronary artery disease however, endothelial dysfunction is manifested as impaired coronary dilatation or even paradoxical vasoconstriction. However, such assessment of the coronary arteries is an invasive and somewhat cumbersome technique and alternative approaches have been developed that correlate well to coronary endothelial function. Ultrasound-based measurement of flow-mediated dilatation (FMD) of the brachial artery utilizes the phenomenon that increased blood flow after temporary noninvasive occlusion of the upper arm leads to shear stress and endothelial release of NO leading to vasodilatation under normal conditions[119,120]. Apart from evaluating basal endothelial function, FMD can be used to test the effect of acute or chronic interventions on endothelial function and as such it is commonly used as a convenient surrogate marker for clinical events[37,121,122]. Furthermore, endothelial function is temporarily impaired by a limited period of ischemia followed by reperfusion[123]. It may therefore serve as a model for studies of ischemia-reperfusion injury. FMD can thus be used to measure the degree of such endothelial ischemia-reperfusion injury and evaluate the effect of interventions directed against it[122-124].

Glucagon-like peptide-1

The incretin hormone glucagon-like peptide-1 (GLP-1) is derived from proglucagon, primarily released by the L-cells of the small intestine, but noteworthy also from neurons in the nucleus of the solitary tract of the brainstem[125]. GLP-1 controls blood glucose levels by regulating the release of insulin and glucagon in response to intake of food. GLP-1 receptor agonists have been increasingly used over the last decade for the treatment of diabetes mellitus and have been shown to decrease all-cause mortality, cardiovascular mortality and the rates of myocardial infarction and stroke[126,127]. However, the effects of GLP-1 receptor agonists are not restricted to the control of blood glucose levels. Animal studies have shown that GLP-1 receptor agonists attenuate ischemia-reperfusion injury[72,74,128]. Furthermore, infusion of the GLP-1 receptor agonist exenatide in non-diabetic patients undergoing primary PCI for STEMI has been demonstrated to confer cardioprotection[75,76] and this effect is irrespective of baseline blood glucose levels, showing that the mechanism is not related to control of acute hyperglycaemia[129]. Interestingly, the release of GLP-1 is modulated by the vagal nerve[71] which is also suggested to be involved in RIC[52], thereby indicating a possible link between those two cardioprotective approaches.

Ticagrelor

Rupture of an atherosclerotic plaque in the acute coronary syndrome (ACS) exposes circulating platelets to the subendothelium[130]. Thereby a complex multifactorial prothrombotic avalanche is triggered including interaction with subendothelial proteins such as von Willebrand factor and collagen, adhesion of platelets to the vessel wall and release of prothrombotic factors from platelet granules resulting in recruitment and reshaping of additional platelets, activation of the coagulation cascade and formation of a growing and potentially occluding vascular thrombus[131] leading to STEMI. Adenosine diphosphate (ADP) is one of the major prothrombotic factors released from platelet granules during this process[132]. ADP is formed by dephosphorylation of adenosine triphosphate (ATP) and stored in dense granules in the platelets. Upon release, ADP interacts with the platelet surface bound G-protein coupled purinergic $P2Y_{12}$ receptor to further promote platelet aggregation, largely through phosphatidylinositol 3-kinase (PI3K) dependent pathways. To counteract the deleterious drive for coronary thrombosis associated with ACS, dual antiplatelet therapy is recommended[133]. In addition to cyclooxygenase inhibition by aspirin, the directly acting reversible $P2Y_{12}$ receptor inhibitor ticagrelor is preferred after showing superiority to clopidogrel in the PLATO trial[134]. Following an acute loading dose, guidelines recommend continuous treatment for one year. Prolonged duration of ticagrelor treatment is considered for certain high-risk individuals. However, this recommendation is primarily based on one large randomized trial showing a modest risk-benefit gain[135]. Importantly, underlying mechanisms for this prolonged protection are poorly understood, but actions beyond platelet inhibition have been suggested[136]. Apart from its antithrombotic $P2Y_{12}$ receptor-dependent properties, ticagrelor is also an inhibitor of adenosine reuptake through the equilibrative nucleoside transporter 1 (ENT1) in the cardiomyocyte cell membrane, resulting in increased extracellular levels of adenosine and thereby adenosine-related pleiotropic cardiovascular effects[137,138]. This is of potential interest as there is evidence for adenosine-induced protection against ischemia-reperfusion injury[139,140]. In fact, ticagrelor has been shown to confer cardioprotection in animal studies of ischemia-reperfusion in an adenosine-dependent manner[141-144]. Whether ticagrelor protects against ischemia-reperfusion injury in humans has not been investigated previously.

Summary

Reperfusion injury is a major contributor to final infarct size in STEMI. As of yet, there is no established therapy directed towards reperfusion injury. The concept of protecting against reperfusion injury through RIC as an adjunct to primary PCI has shown promising results in early studies, but the overall picture is not entirely conclusive and the underlying mechanisms are not fully known. Additional trials based on robust endpoints are needed to establish the role for RIC in STEMI. Further exploration of the underlying mechanisms may give the prerequisites to take full advantage of the underlying therapeutic potential.

AIMS

The overall aim of this thesis was to evaluate the effect of RIC in patients with STEMI and to explore the underlying mechanisms of RIC in humans. The specific aims were to investigate if:

1. RIC confers cardioprotection when provided in addition to primary PCI in patients with STEMI (Study I and II)

2. The effect of RIC on endothelial ischemia-reperfusion injury in humans involves activation of the GLP-1 receptor (Study III)

3. Treatment with ticagrelor is associated with protection against endothelial ischemia-reperfusion injury and improved endothelial function in patients with a previous ACS (Study IV)

METHODS

All studies were approved by the regional ethics committee in Stockholm and conducted according to the Declaration of Helsinki[145].

Study subjects

Studies I and II

Consecutive patients arriving to any of the including hospitals (Karolinska University Hospital Solna, Karolinska University Hospital Huddinge, Södersjukhuset and Danderyd Hospital, all in Stockholm, Sweden) with signs and symptoms of anterior STEMI were screened for participation. Inclusion criteria were chest pain indicating myocardial ischemia with a duration >30 minutes and <6 hours, ST-elevation >0.1 mV (>0.2 mV in V2-V3) in two contiguous leads (V1-V6) and age over 18 years. Exclusion criteria were previous myocardial infarction, left bundle branch block, previous coronary artery bypass grafting, cardiac arrest, severe claudication, atrial fibrillation, treatment with glibenclamide or cyclosporine, any condition that may interfere with the possibility for the patient to comply with the study protocol or PCI not performed. After informed oral consent patients were entered into the study. Completive written consent was acquired within 24 hours.

Study III

Healthy male adults were eligible for the study and recruited through posts on announcement boards at Karolinska Institutet and through telephone calls to previous participants in medical trials. After oral and written information, written consent was acquired and patients were entered into the study.

Study IV

Consecutive eligible male patients with a recent ACS awaiting their one-year clinical follow-up visit at Karolinska University Hospital Solna, Karolinska University Hospital Huddinge or Danderyd Hospital were identified from the follow-up part of the SWEDEHEART (Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapy) registry[146]. The registry was at the time containing all patients age ≤75 years treated for an ACS in Sweden. Inclusion criteria were ACS within one year and ongoing ticagrelor treatment planned for discontinuation according to clinical guidelines. Patients with diabetes mellitus were excluded from the study. Patients were contacted through telephone calls and after oral and written information, written consent was acquired and patients were entered into the trial.

Study design

Studies I and II

The trial was performed as a multicenter randomized controlled trial with blinded evaluation. After informed consent, eligible patients fulfilling inclusion but not exclusion criteria were randomized 1:1 to receive RIC or sham procedure. Randomization was computer-generated in blocks of eight. RIC was performed by inflating a blood pressure cuff around the left thigh to 200 mmHg (or 20 mmHg above SBP if SBP was above 180 mmHg) for five minutes and

then deflating, with a new cycle starting five minutes after deflation. RIC started immediately after randomization. At least one cycle was to be completed before first coronary balloon inflation and another four cycles thereafter **(Figure 5)**.

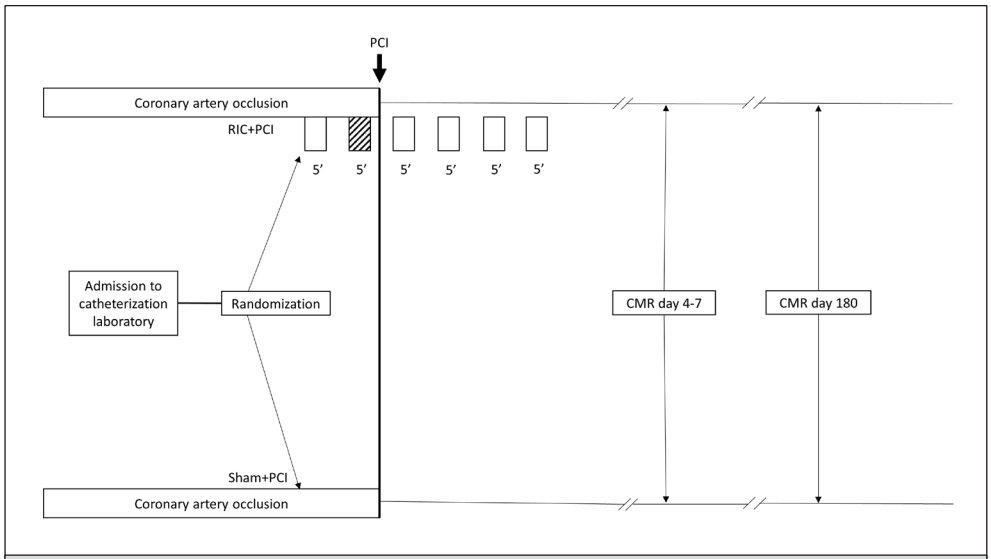

Figure 5. Protocol for Studies I and II. Dashed square indicates additional conditioning cycles performed before PCI if time allowed. CMR = cardiac magnetic resonance imaging; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Inflations of the blood pressure cuff were performed through an automated device (PeriVasc Cuff Unit, EBIDA, Göteborg, Sweden). Sham procedure was performed by placing the blood pressure cuff around the left thigh, without inflating it.

PCI was performed according to local guidelines. Double antiplatelet therapy was given with aspirin (500 mg) and ticagrelor (180 mg) or clopidogrel (600 mg) in the ambulance or at arrival at the catheterization laboratory. Additional medication and interventional considerations were according to clinical guidelines and at the discretion of the PCI operator. Venous blood samples for analyses of high sensitivity troponin T were collected on admission, after six and twelve hours and then every eight hours until 44 hours from inclusion.

Patients underwent a first CMR (see below for detailed protocol) four to seven days after the PCI and a second CMR six months later. Follow-up of clinical data up to three years after the event was through patient charts, registries and telephone calls to patients.

Study III

Examinations took place in the morning. Subjects were overnight fasting and refraining from tobacco products. For the main study, subjects were examined with three different protocols (A-C) on three separate occasions with at least six days between visits **(Figure 6)**.

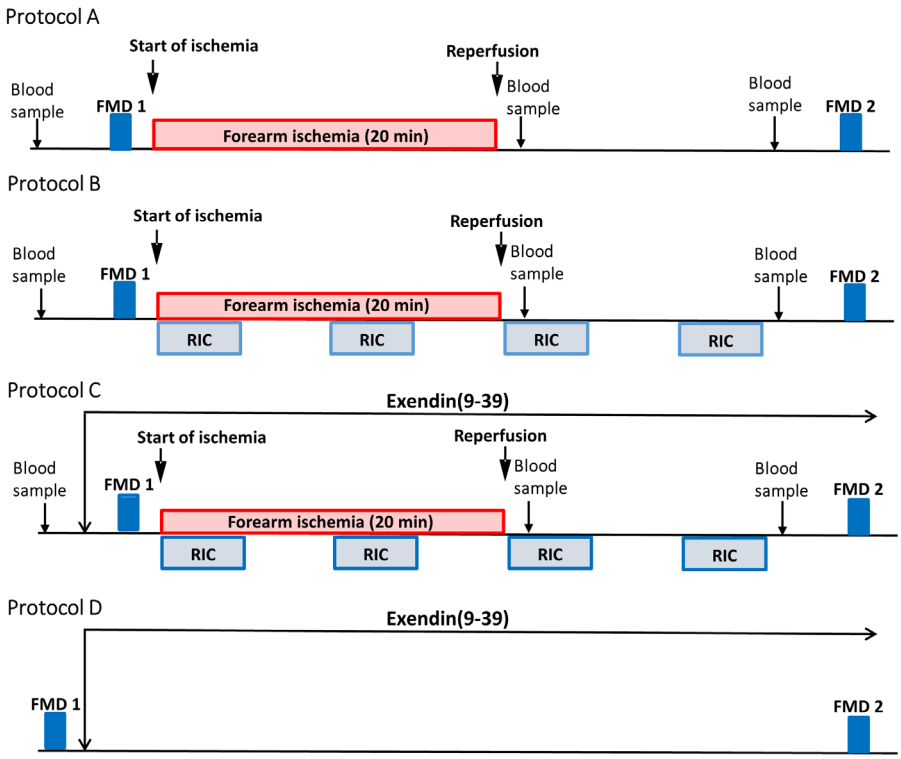

Figure 6. Protocol for Study III. Protocol A = Ischemia-reperfusion. Protocol B = Ischemia-reperfusion + RIC. Protocol C = Exendin(9-39) + ischemia-reperfusion + RIC. Protocol D = Exendin(9-39). FMD = flow-mediated dilatation; GLP-1 = glucagon like peptide 1; RIC = remote ischemic conditioning.

The order of the protocols was randomized for each subject. In protocol A, FMD was examined in the left brachial artery (see below for detailed description) before and after 20 minutes of ischemia followed by 20 minutes of reperfusion to measure the degree of endothelial impairment induced by ischemia-reperfusion. In protocol B, FMD was tested before and after 20 minutes of ischemia and 20 minutes of reperfusion with simultaneous RIC to evaluate its conditioning effect. In protocol C, FMD was performed before and after 20 minutes of ischemia and 20 minutes of reperfusion with simultaneous RIC and intravenous infusion of the GLP-1 receptor antagonist exendin(9-39) (Bachem, Bubendorf, Switzerland) to explore the impact of GLP-1 receptor inhibition. In an additional protocol (D), FMD was examined before and after 40 minutes infusion of exendin(9-39) to evaluate its effect on FMD per se (no ischemia-reperfusion was performed in this protocol).

Endothelium-independent vasodilatation (EIVD) was evaluated at the end of each protocol by performing an additional FMD-measurement after sublingual administration of 0.4 mg nitroglycerine.

Ischemia-reperfusion was performed by inflating a blood pressure cuff around the left upper arm to 200 mmHg for 20 minutes and then deflating it for 20 minutes of reperfusion.

RIC was performed by inflating a blood pressure cuff around the left thigh to 200 mmHg for five minutes and then deflating, with a new cycle starting five minutes after deflation. Four such cycles (two during ischemia and two during reperfusion) were performed **(Figure 7)**.

Figure 7. Setup for Study III. FMD was evaluated by ultrasound in the left brachial artery after inflation (200 mmHg for five minutes) and deflation of a blood pressure cuff around the upper part of the left forearm. Ischemia-reperfusion was achieved by inflating a blood pressure cuff around the left upper arm to 200 mmHg for 20 minutes followed by 20 minutes reperfusion by deflating the cuff. RIC was performed by inflating a blood pressure cuff around the left thigh to 200 mmHg for five minutes and then deflating, with a new cycle starting five minutes after deflation. FMD = flow-mediated dilatation; RIC = remote ischemic conditioning.

Exendin(9-39) was administered through a cannula in a superficial vein in the right antecubital fossa. A bolus of 7500 pmol/kg was given 10 minutes before the first FMD measurement followed by infusion of 500 pmol/kg/min throughout the protocol. This dose is known to antagonize the effect of GLP-1 in humans[147,148].

Venous blood samples for analysis of plasma GLP-1 were collected before forearm ischemia and one minute and 16 minutes after start of reperfusion. Venous blood samples for analysis of plasma insulin were collected in EDTA tubes before baseline FMD and at the end of reperfusion.

Study IV

Examinations took place in the morning. Patients were overnight fasting and refraining from tobacco products, but were instructed to take their morning medication (1-2 hours before examinations). Each patient was examined on three occasions with identical protocols **(Figure 8)**.

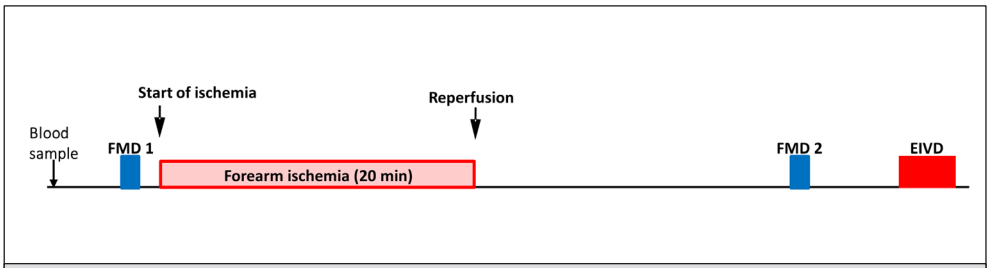

Figure 8. Protocol for Study IV. An identical protocol was used for all three visits. The first visit was at the end of the planned 12-month period of ticagrelor treatment, after which the drug was discontinued. The second and third visits were two and four week after discontinuation of ticagrelor treatment. EIVD = endothelium-independent vasodilatation; FMD = flow-mediated dilatation.

The first visit (A) coincided with the last day of the twelve month ticagrelor treatment after ACS, after which the drug was discontinued according to clinical indication. Another two visits (B and C) followed with an interval of two to four weeks between each visit. FMD was examined in the left brachial artery before and after 20 minutes of arm ischemia followed by 20 minutes of reperfusion as described for Study III above.

EIVD was evaluated at the end of each protocol by performing an additional FMD-measurement after sublingual administration of 0.4 mg nitroglycerine. Venous blood samples were collected at the beginning of each visit for analyses of platelet inhibition.

CMR

CMR examinations were performed at the Karolinska University Hospital using a 1.5 T Siemens Magnetom Aera scanner (Siemens Healthcare, Erlangen, Germany) in Studies I and II. A gadolinium-based contrast agent (Gd-DTPA, 0.2 mmol/kg, Dotarem, Guerbet, France) was used. ECG-gated images in 12-14 short axis views (eight mm thickness, two mm gap) and in the two-, three- and four-chamber views were acquired during breath-holds. For determination of MaR, early contrast-enhanced steady state free precession images were obtained. For quantification of infarct size, late gadolinium-enhancement (LGE) images were collected 15 to 20 minutes after contrast injection using an inversion recovery gradient echo sequence. Segment v1.9 R3967 (http://segment.heiberg.se) was used for offline analyses of images[149]. Epi- and endocardial borders were manually outlined on short axis stacks of early contrast-enhanced steady state free precession and LGE images. MaR was manually outlined on early contrast-enhanced steady state free precession sequences[150]. The automated Otsu quantification method[151] with weighted approach[152] and manual adjustments where needed was used to determine infarct size and microvascular obstruction. Two observers blinded to randomization performed individual analyses of CMR images, followed by final co-evaluation to reach consensus. Intra-observer variability for MaR and infarct size determinations were 0.9 ± 2.3% and 0.5 ± 1.6% (bias and SD), respectively.

Angiographic evaluation

TIMI blood flow[153] in the infarct related artery before and after coronary intervention and the presence of collaterals according to the Rentrop classification[154] was evaluated by the PCI operator. Two experienced interventional cardiologists blinded to the randomization evaluated MaR according to BARI (Bypass Angioplasty Revascularization Investigation)[93] and modified APPROACH (Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease)[94] scores.

FMD

FMD was performed with a Vivid E9® (GE, Waukesha, Wisconsin, USA) and an 11MHz (output 12MHz) linear-array transducer mounted on a flexible arm for stabilization. End-diastolic baseline artery diameter was recorded every 3 seconds for 1 minute and a mean value was calculated. A blood pressure cuff was inflated to 200 mmHg for five minutes around the upper part of the left forearm and the deflated. The brachial diameter was continuously recorded for three minutes after deflation and a mean diameter was calculated from the three frames with maximal dilatation at end-diastole[155]. Diameter was measured intima-intima and FMD was calculated as: diameter following cuff deflation – baseline diameter ⁄ baseline diameter × 100. Peak blood flow velocity during hyperemia was recorded with pulsed Doppler and a mean from three cardiac cycles was calculated. A technician blinded to the protocol performed evaluations. Intra-observer variability for FMD was 0.1 ± 3.9 (bias and SD).

Biochemical analysis

Blood samples for the analysis of plasma GLP-1 were immediately placed in tubes containing the dipeptidyl peptidase 4 inhibitor diprotin (final concentration 0.1 mmol/L) to avoid cleavage. Plasma GLP-1 was analyzed with ELISA No EZGLP1T (Merck), measuring total GLP-1 (both the active form (7-36) and fragments of GLP-1)[156]. The same lot was used for all samples. The intra-assay variation is <5%. Plasma insulin was analyzed by electrochemiluminescence immunoassay. Blood samples for the analysis of platelet inhibition were collected in Hirudin-containing tubes and analyzed within two hours using impedance aggregometry (Multiplate, Roche Diagnostics)[157]. The effect of ticagrelor and aspirin was evaluated with the ADP-test (platelet function after stimulation of platelet adenosine diphosphate receptors) and ASPI-test (platelet function stimulated by arachidonic acid) respectively. Simultaneous analysis was performed to confirm platelet counts above 50 x 10^9/L (using EDTA containing tubes).

Statistical analysis

GraphPad Prism version 6.05 for Windows (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis.

Studies I and II

The primary endpoint of Study I was myocardial salvage index (MSI) defined as 100 × (MaR-infarct size)/MaR. Power size calculation assuming a relative reduction of infarct size in

relation to MaR of 30% (based on a relative reduction of 50% of infarct size in relation to MaR in patients with LAD occlusions in a previous study of RIC[38]) and an SD of 0.66 using log transformed data (based on a previous study, quantifying infarct size and MaR with CMR[158]), 88 patients were needed to achieve 80% power and a statistical level of 0.05. To compensate for non-parametric tests, dropouts and poor CMR image quality, an additional 30% of patients were planned to be included. Secondary outcomes included major adverse cardiovascular events within 30 days, infarct size according to high sensitivity troponin T and infarct size on CMR according to absolute values. As Study II was a follow-up study of Study I, it did not have a separate power calculation. Its primary endpoint was MSI at 6 months (using infarct size from the second CMR and MaR from the first CMR). Secondary CMR-based outcomes were final absolute infarct size and left ventricular ejection fraction (LVEF). Secondary clinical endpoints were analyzed up to three years after the event and included cardiac mortality, MACCE (composite of all-cause mortality, myocardial infarction, readmission for heart failure, ischemic stroke (including transient ischemic attack) and target lesion revascularization) and all the individual components of MACCE. Non-parametric Mann-Whitney U-test was used for continuous variables and Fisher's exact test for categorical variables. Intra-observer variability was evaluated with Bland-Altman analysis. Data are presented as medians and interquartile ranges if not otherwise indicated.

Study III

The primary endpoint was change in FMD from baseline following ischemia-reperfusion. Assuming a relative improvement in FMD of 30% (based on our own pilot studies), 12 subjects were needed to achieve 80% power at a significance level of 0.05. To compensate for examinations with poor image quality and for possible dropouts, fourteen subjects were recruited. Normality of data was tested using D'Agostino and Pearson normality test. One-way ANOVA was used for comparison of treatment effect and two-way ANOVA for comparison of FMD and plasma levels of GLP-1 between baseline and after ischemia-reperfusion and between groups. Intra-observer variability was tested with Bland-Altman analysis. Data are presented as means ± SEM if not otherwise indicated.

Study IV

The primary endpoint of the study was change in FMD from baseline to after ischemia-reperfusion. Based on our previous studies of protection against endothelial ischemia-reperfusion injury[159,160], an absolute improvement in FMD of 1.3 % was estimated. Thus, with a standard deviation of 1.8%, 15 patients were needed for 80 % power at a significance level of 0.05. To compensate for possible dropouts and for examinations with poor image quality, 20 volunteers were recruited. Normality of data was tested using D'Agostino and Pearson normality test. Ischemia-reperfusion induced change in FMD at each visit was analyzed with paired t-test. Platelet inhibition, basal endothelial function and endothelial ischemia-reperfusion injury was compared between visits with one-way ANOVA and Tukey's multiple comparisons post-hoc test was used where appropriate. Intra-observer variability was tested with Bland-Altman analysis. Data are presented as means ± SEM or medians and interquartile ranges if not otherwise indicated.

RESULTS

Study groups

Studies I and II

Out of 594 consecutive patients screened for participation, 150 fulfilled the entry criteria and could be randomized to RIC or sham procedure (**Figure 9**).

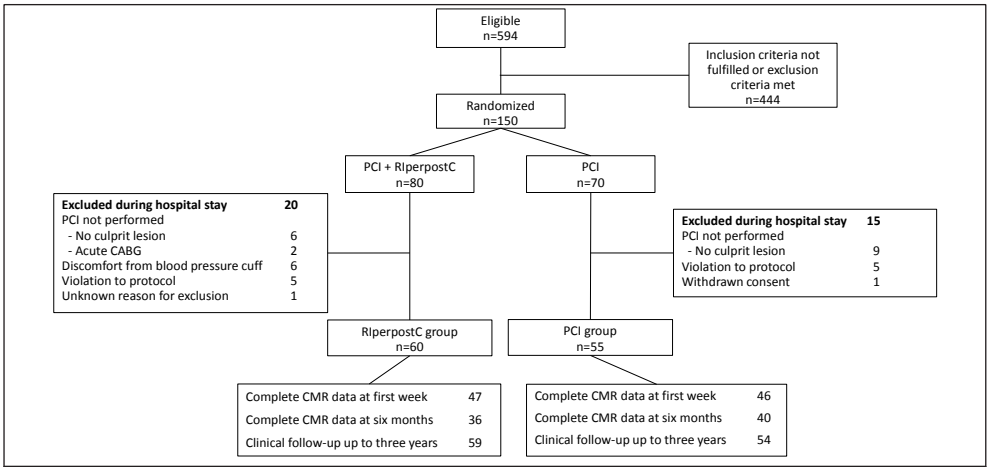

Figure 9. Patient flow chart in Studies I and II. CABG = coronary artery bypass grafting; CMR = cardiac magnetic resonance; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Thirty-five patients were excluded during hospital stay, leaving 115 patients in the per-protocol analysis of Study I. For the long term follow-up of Study II, survival data were available for 113 patients as one patient withdrew consent and one patient was lost to follow-up. 93 patients completed a first CMR examination of sufficient quality for analysis of the primary endpoint of Study I and 76 of those patients underwent a second CMR examination after six months for analysis of the primary endpoint of Study II **(Figure 10)**.

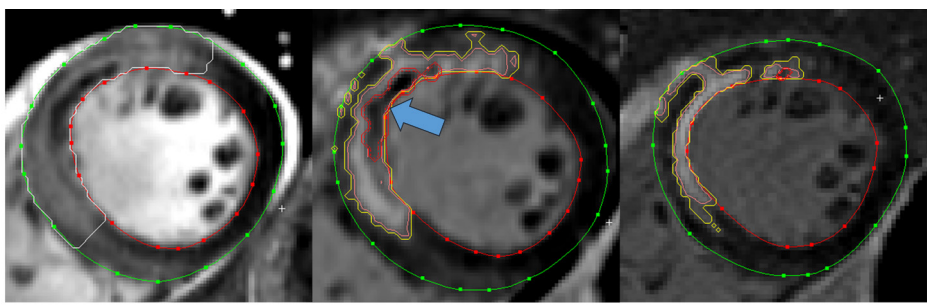

Figure 10. Anterior myocardial infarction on CMR imaging in Studies I and II. MaR with contrast-enhanced steady state free precession from CMR imaging 4-7 days after STEMI (left). Myocardial scar with LGE from CMR imaging 4-7 days after STEMI (center). Myocardial scar with LGE from CMR imaging 6 months after STEMI (right). Blue arrow indicates microvascular obstruction present on early CMR but not on follow-up examination. CMR = cardiac magnetic resonance; LGE = late gadolinium enhancement; MaR = myocardium at risk; STEMI = ST-elevation myocardial infarction.

Baseline and periprocedural characteristics were overall well balanced between the groups (**Table III and Table IV**), although the RIC group had a notably higher prevalence of smokers and occlusions of the proximal LAD.

Table III. Baseline characteristics of all patients in Studies I and II. Data are presented as median and interquartile range or number of subjects and percentages. Significant differences were analyzed using Mann-Whitney U-test and Fisher's exact test. PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Variables	PCI (n=55)	RIC+PCI (n=60)
Age, years	63 (57-69)	62 (53-68)
Men	52 (95)	54 (90)
Symptom to balloon time, min	158 (115-252)	145 (122-203)
Current smoker	16 (29)	30 (50)
Current treatment for hypertension	15 (27)	12 (20)
Current treatment for dyslipidemia	3 (5)	4 (7)
Previously known diabetes mellitus	6 (11)	6 (10)
Killip class 1	52 (95)	54 (90)

Table IV. Periprocedural characteristics of patients included in analysis of MSI on CMR at day 4-7 in Study I. Data are presented as median and interquartile range or number of subjects and percentages except for number of remote ischemia cycles performed which is presented as median and total range. Significant differences were analyzed using Mann-Whitney U-test and Fisher´s exact test. CMR = cardiac magnetic resonance; GP-IIb/IIIa = glycoprotein IIb/IIIa; LAD = left anterior descending artery; MSI = myocardial salvage index; PCI = percutaneous coronary intervention; RCA = right coronary artery; RIC = remote ischemic conditioning; TIMI = Thrombolysis In Myocardial Infarction.

Variables	PCI (n=46)	RIC+PCI (n=47)
Periprocedural medication		
Ticagrelor	43 (93)	46 (98)
Clopidogrel	27 (59)	24 (51)
Bivalirudin	41 (89)	41 (87)
GP-IIb/IIIa inhibitor	5 (11)	3 (6)
Morphine	42 (91)	40 (85)
Angiographic and interventional variables		
Radial access	39 (85)	38 (81)
Culprit vessel		
LAD	42 (91)	46 (98)
Proximal LAD	11 (24)	18 (38)
Circumflex	2 (4)	0 (0)
RCA	2 (4)	1 (2)
TIMI 0-1 flow on presentation	42 (91)	39 (83)
TIMI 2-3 flow on presentation	4 (9)	8 (17)
Rentrop score 0	38 (83)	36 (77)
Rentrop score 1	5 (11)	6 (13)
Rentrop score 2	4 (9)	5 (11)
Thrombus aspiration performed	8 (17)	10 (21)
Direct stenting performed	6 (13)	9 (19)
Number of remote ischemia cycles performed	N/A	7 (5-11)

Study III

Fourteen volunteers underwent protocol A-C in the main part of the study. Two of those subjects were excluded due to insufficient image quality of the FMD registration. Eleven subjects (nine of which had also participated in protocol A-C) were examined with protocol D. Study subjects had a mean age of 30.5 (range 19-48) years and a body mass index of 24 ± 0.7. Mean fasting glucose was 5.1 ± 0.1 and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) 1.5 ± 0.3. All study subjects self-assessed their exercise capacity as normal or good. Two subjects were smokers and one subject reported regular intake of dietary supplements (vitamin C and omega 3). The examination protocols were well tolerated by all subjects.

Study IV

Twenty patients with a mean age of 60 (range 40-74) years were recruited. Baseline characteristics of the patients are shown in **Table V**. Examinations with insufficient image quality or motion artefacts were excluded. Seventeen patients at each visit remained for final analysis of FMD.

Table V. Baseline characteristics of patients in Study IV. Medians and interquartile ranges or numbers and percentages. n=20. BMI = body mass index; CRP = C-reactive protein; DBP = diastolic blood pressure; GFR = glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SBP = systolic blood pressure; ACE-I = angiotensin converting enzyme-inhibitor; ARB = angiotensin II receptor blocker; TG = triglycerides.

Variables	**Study group**
Age (years)	60 (40-74)
BMI (kg/m^2)	28.7 (25.8-29.7)
SBP (mmHg)	130 (120-144)
DBP (mmHg)	75 (70-83)
HbA1c (mmol/mol)	36 (34-39)
GFR (ml/min/1.7)	78 (69-87)
hs-CRP (mg/l)	0.56 (0.36-0.95)
fP-LDL (mmol/l)	1.4 (1.1-1.7)
fP-Cholesterol (mmol/l)	3.3 (2.6-3.4)
fP-TG (mmol/l)	1.0 (0.6-1.2)
Current smokers	3 (15)
Previous smokers	6 (30)
Aspirin	20 (100)
Statin	20 (100)
Betablocker	17 (85)
ACE-I	9 (45)
ARB	5 (25)

Study outcomes

Effect of RIC on myocardial infarct size in patients with anterior STEMI (Studies I and II)

MSI based on infarct size and MaR from CMR was the primary endpoint to evaluate the effect of RIC in STEMI. RIC did not significantly affect MSI as measured by CMR performed on day 4-7 (RIC group 48.5% vs control group 49.2%; p = 0.85) (**Figure 11)** or after six months (RIC group 67.1% vs control group 67.6%; p = 0.93) **(Figure 12**).

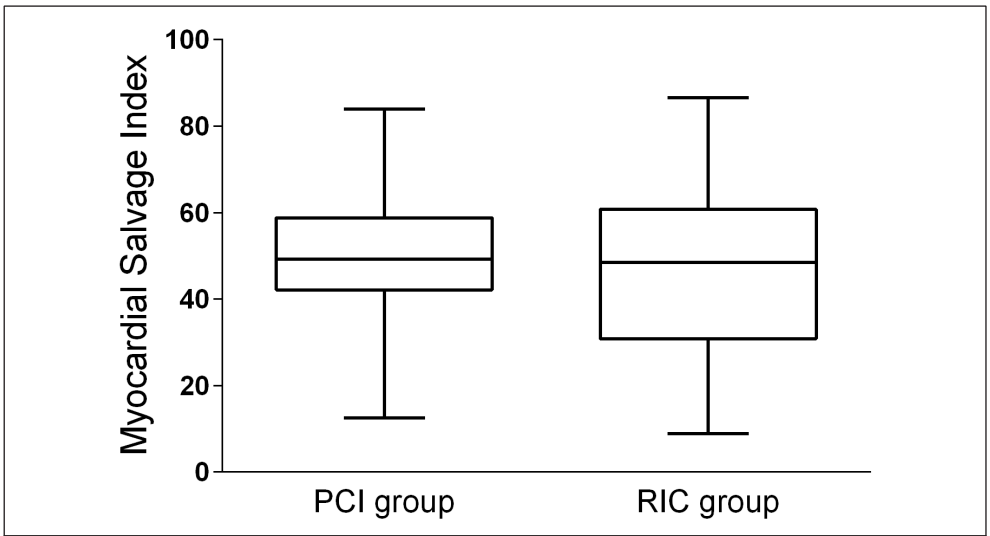

Figure 11. MSI in Study I. MSI in the PCI group and the RIC + PCI group. Line and box mark median and interquartile range. Whiskers show min and max. MSI = myocardial salvage index; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

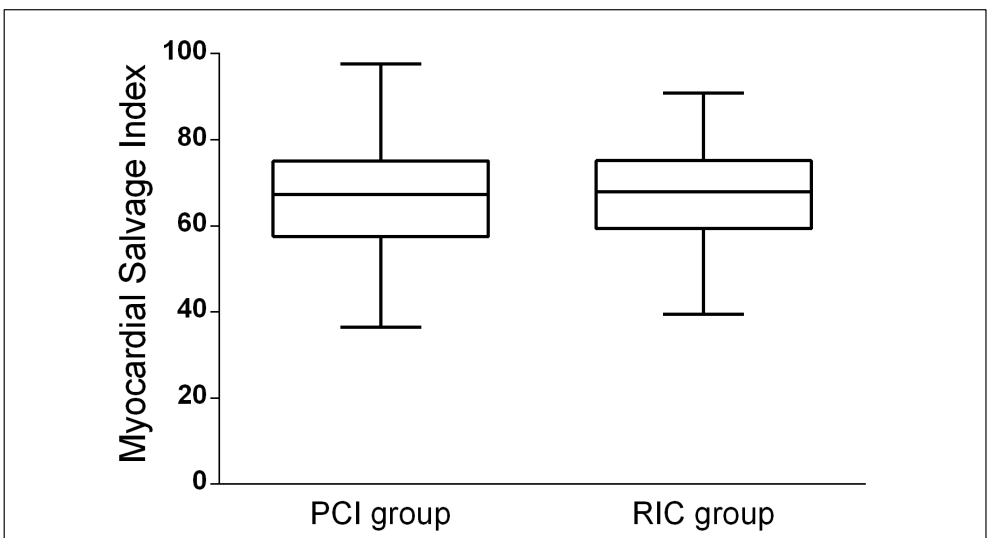

Figure 12. MSI in Study II. MSI in the PCI group and the RIC + PCI group. Line and box mark median and interquartile range. Whiskers show min and max. MSI = myocardial salvage index; PCI = percutaneous coronary intervention. RIC = remote ischemic conditioning.

Likewise, absolute infarct size and left ventricular function on acute and follow-up CMR was not different between the study groups. MaR was significantly larger in the RIC group as measured by CMR (43.1% vs 37.0% of the left ventricle; P = 0.03), but not when evaluated by BARI and modified APPROACH scores. Angiographic and CMR data are shown in **Table VI and Table VII**. Troponin T peak value (RIC group 5025 ng/l vs. control group 5350 ng/l; p = 0.73) and 44 h area under the curve (RIC group 55620 vs. control group 61560; p = 0.09) were not significantly affected by RIC. Exploratory subgroup analyses based on symptom to balloon time, culprit vessel, TIMI-flow grade in culprit vessel at presentation, number of RIC cycles or smoking status did not result in significant differences between study groups (**Table VIII**).

Table VI. Parameters from acute coronary angiography and CMR on day 4-7 in Study I. Data are presented as median and interquartile range or number of subjects and percentages. Significant differences were analyzed using Mann-Whitney U-test and Fisher's exact test. BSA = body surface area; CMR = cardiac magnetic resonance; LV = left ventricle; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MSI = myocardial salvage index; RIC = remote ischemic conditioning.

Variables	PCI (n=46)	RIC+PCI (n=47)	p
Infarct size (% of LV)	17.9 (13.4-25.0)	20.6 (14.1-31.7)	0.26
MaR from CMR (% of LV)	37.0 (30.8-44.1)	43.1 (35.4-49.7)	0.03
MSI	49.2 (42.1-58.8)	48.5 (30.9-60.8)	0.85
LVEF (%)	50.1 (45.5-53.9)	48.1 (41.0-55.3)	0.24
LVEDV indexed for BSA (ml/m^2)	89.4 (81.5-97.1)	90.0 (79.2-104.2)	0.74
LV mass indexed for BSA (g/m^2)	78.0 (70.7-87.2)	81.1 (72.2-89.2)	0.62
Left ventricular thrombus	5 (11)	7 (15)	0.76
TIMI 3 flow after PCI	44 (96)	47 (100)	0.24
BARI score (% of LV)	33 (28-43)	38 (29-43)	0.28
Modified APPROACH score (% of LV)	32 (28-47)	32 (30-47)	0.30

Table VII. Parameters from CMR at six months in Study II. Median and interquartile range. Differences were analyzed with Mann-Whitney U-test. BSA = body surface area; LV = left ventricle; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; MSI = myocardial salvage index; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Variables	PCI (n=40)	RIC+PCI (n=36)	P
Infarct size (% of LV)	11.6 (8.5-17.0)	14.2 (10.3-18.7)	0.23
Recovery of infarct size (% of LV)	6.6 (3.4-9.0)	7.5 (5.0-13.1)	0.31
MSI	67.3 (57.6-75.1)	67.9 (59.4-75.2)	0.93
LVEF (%)	56.5 (50.9-64.6)	53.2 (47.0-64.8)	0.29
Recovery of LVEF (% of LV)	8.5 (1.9-12.0)	7.3 (1.5-12.3)	0.45
LVEDV indexed for BSA (ml/m^2)	93.6 (79.9-108.1)	93.6 (78.0-106.6)	0.73
LVESV indexed for BSA (ml/m^2)	40.2 (28.3-51.9)	43.0 (29.3-55.6)	0.67
LV mass indexed for BSA (g/m^2)	76.9 (69.1-88.1)	79.7 (68.8-90.5)	0.61

Table VIII. Myocardial salvage index in subgroups in Study I. Data are presented as median and interquartile range. Significant differences were analyzed using Mann-Whitney U-test. RIC = remote ischemic conditioning. N/A=not applicable.

Subgroup	PCI	RIC+PCI	p
Non-smokers **n**	49.7 (38.1-60.0) 32	45.4 (30.6-54.9) 26	0.37
LAD culprit vessel **n**	49.7 (42.7-59.2) 43	48.5 (30.3-61.3) 45	0.56
Symptom to balloon time ≤145 min **n**	48.7 (40.7-59.9) 22	54.4 (30.3-64.7) 25	0.95
Symptom to balloon time >145 min **n**	49.7 (41.6-58.2) 24	46.9 (35.6-59.5) 22	0.65
TIMI-flow grade 0 **n**	48.3 (35.8-55.5) 34	46.3 (28.9-60.6) 36	0.81
≤6 conditioning cycles **n**	N/A	52.7 (32.8-71.7) 18	N/A
≥7 conditioning cycles **n**	N/A	52.3 (39.3-81.7) 27	N/A

Effect of RIC on clinical outcomes in patients with anterior STEMI (Studies I and II)

No patients died during primary PCI. Four patients in the RIC group and five patients in the control group died during the follow-up period of three years. Causes of death were myocardial infarction (four patients), cardiac arrest, heart failure, intracerebral hemorrhage, sepsis and esophageal cancer. Cardiac mortality, MACCE or the individual components of MACCE did not differ between the groups at 30 days **(Table IX)**, six months **(Table X)** or three years **(Table XI**).

Table IX. Clinical outcomes at 30 days in Study I. Number of patients and percentages. Differences were analyzed with Fisher's exact test. MACCE = major adverse cardiac and cerebrovascular events; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Variables	PCI (n=55)	RIC+PCI (n=60)	P
MACCE	4 (7)	1 (2)	0.19
All-cause mortality	2 (4)	1 (2)	0.61
Cardiac mortality	2 (4)	1 (2)	0.61
Myocardial reinfarction	0 (0)	0 (0)	1.00
Target lesion revascularization	1 (2)	0 (0)	0.48
Readmission for heart failure	0 (0)	0 (0)	1.00
Ischemic stroke/transient ischemic attack	0 (0)	0 (0)	1.00
Use of iv diuretics during hospital stay	11 (21)	20 (34)	0.14

Table X. Clinical outcomes at 6 months in Study II. Number of patients and percentages. Differences were analyzed with Fisher's exact test. MACCE = major adverse cardiac and cerebrovascular events; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Variables	PCI (n=54)	RIC+PCI (n=59)	P
MACCE	4 (7)	5 (9)	1.00
All-cause mortality	4 (7)	2 (3)	0.42
Cardiac mortality	3 (6)	1 (2)	0.35
Myocardial infarction	1 (2)	1 (2)	1.00
Target lesion revascularization	0 (0)	1 (2)	1.00
Readmission for heart failure	0 (0)	2 (4)	0.50
Ischemic stroke/transient ischemic attack	1 (2)	0 (0)	0.48

Table XI. Clinical outcomes at 3 years in Study II. Number of patients and percentages. Differences were analyzed with Fisher's exact test. MACCE = major adverse cardiac and cerebrovascular events; PCI = percutaneous coronary intervention; RIC = remote ischemic conditioning.

Variables	PCI (n=54)	RIC+PCI (n=59)	P
MACCE	9 (17)	8 (14)	0.79
All-cause mortality	5 (9)	4 (7)	0.73
Cardiac mortality	3 (6)	3 (5)	1.00
Myocardial infarction	3 (5)	2 (4)	0.66
Target lesion revascularization	1 (2)	2 (4)	1.00
Readmission for heart failure	1 (2)	3 (6)	0.62
Ischemic stroke/transient ischemic attack	1 (2)	0 (0)	0.49

Impact of exendin(9-39) on the effect of RIC on endothelial ischemia-reperfusion injury (Study III)

The degree of endothelial dysfunction in response to ischemia-reperfusion was determined in protocol A as the change in FMD from baseline. Ischemia-reperfusion impaired endothelial function (FMD at baseline 4.7 ± 0.8% vs. 1.5 ± 0.4% after ischemia-reperfusion; $p < 0.01$) (**Figure 13**).

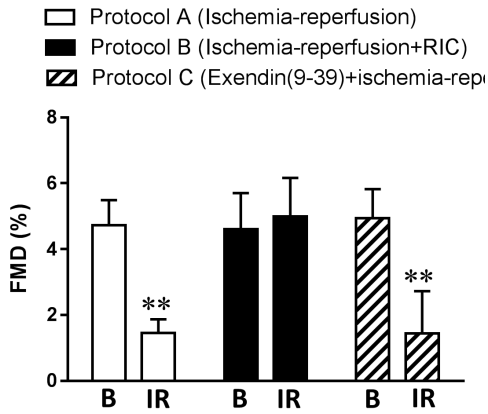

Figure 13. Impairment of endothelial function following ischemia-reperfusion. FMD at baseline and following ischemia-reperfusion in protocol A, B and C. Mean values with 95% CI. Significant differences from baseline FMD within each protocol are depicted. **p<0.01; B = baseline; FMD = flow-mediated dilatation; I/R = ischemia-reperfusion; RIC = remote ischemic conditioning.

In protocol B, RIC during ischemia-reperfusion protected against this attenuation of FMD (baseline 4.6 ± 1.1% vs. 5.0 ± 1.2% after ischemia-reperfusion). In protocol C, simultaneous intravenous infusion of the GLP-1 receptor antagonist exendin(9-39) abolished the protection provided by RIC (FMD at baseline 4.9 ± 0.9% vs. 1.5 ± 1.3% after ischemia-reperfusion; p <0.01). Consequently, FMD after ischemia-reperfusion was significantly greater in protocol B (ischemia-reperfusion + RIC) than in protocol A (control ischemia-reperfusion) (p <0.05) and protocol C (exendin(9-39) + ischemia-reperfusion + RIC) (p <0.05). There was no difference in baseline brachial artery diameter or maximal flow rate during hyperemia between the protocols A-C **(Table XII**). EIVD of the brachial artery was not significantly different between the examinations. Plasma levels of insulin and GLP-1 were not significantly altered in any of the protocols (**Table XIII).**

Table XII. Brachial artery diameter and peak flow in Study III. Means and SEM. IR = ischemia-reperfusion; RIC = remote ischemic conditioning.

	Protocol A		**Protocol B**		**Protocol C**	
Variables	Before IR	After IR	Before IR	After IR	Before IR	After IR
Baseline diameter (mm)	3.6 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.5 ± 0.1	3.7 ± 0.1
Vpeak (cm/s)	58 ± 6	67 ± 5	66 ± 5	69 ± 5	61 ± 6	62 ± 4

Table XIII. Plasma GLP-1 and insulin levels in Study III. Plasma GLP-1 and insulin levels before ischemia and during reperfusion. Values for GLP-1 are pmol/L and for insulin mIE/L. n=12. Means and SEM. GLP-1 = glucagon like peptide-1.

	Protocol A	**Protocol B**	**Protocol C**
Plasma GLP-1			
- Baseline	13.5 ± 3.1	11.8 ± 2.9	11.1 ± 2.9
- At reperfusion	14.2 ± 3.4	14.1 ± 3.0	13.6 ± 3.1
- 16 min after reperfusion	13.8 ± 3.5	12.3 ± 3.0	10.0 ± 2.2
Plasma insulin			
- Baseline	6.4 ± 1.2	5.9 ± 0.7	6.4 ± 0.9
- 16 min after reperfusion	5.3 ± 0.9	7.0 ± 1.5	5.5 ± 0.6

Effect of exendin(9-39) on endothelial function (Study III)
There was no effect of exendin(9-39) *per se* on basal endothelial function (FMD 4.1 ± 0.3% at baseline vs. 4.2 ± 0.5% after 40 minutes infusion), baseline brachial diameter (3.3 ± 0.1 vs. 3.4 ± 0.1 mm) or maximal blood flow (57 ± 4.9 vs. 62 ± 4.8 cm/s). Furthermore, exendin(9-39) did not affect plasma glucose.

Effect of ticagrelor on endothelial function and ischemia-reperfusion injury (Study IV)
The effect of chronic ticagrelor treatment on basal endothelial function was evaluated by comparison of basal FMD between the visits. Chronic ticagrelor treatment was not associated with better basal endothelial function compared to after its discontinuation (Visit A: 3.2 ± 0.7 %; Visit B: 4.6 ± 0.8 %; Visit C: 2.3 ± 0.4 %; p=0.07). Ischemia-reperfusion caused significant impairment of endothelial function at all three visits. (Absolute change in FMD at Visit A: -3.0 (p<0.001), Visit B: -1.9 (p=0.03), Visit C: -1.9 (p<0.0001)). Ticagrelor did not confer protection against endothelial ischemia-reperfusion injury (p=0.39 for comparison between visits of absolute change in FMD after ischemia-reperfusion). (**Figure 14**).

The degree of endothelial impairment in response to ischemia-reperfusion did not differ significantly between the examinations. Baseline brachial artery diameter, maximal flow rate during hyperemia and EIVD of the brachial artery was not significantly different between the examinations (**Table XIV**). Trc-ADP confirmed a marked impairment of platelet function during ticagrelor treatment compared to after its discontinuation. Trc-ASPI was slightly lower at the first visit compared to the two following **(Table XV).**

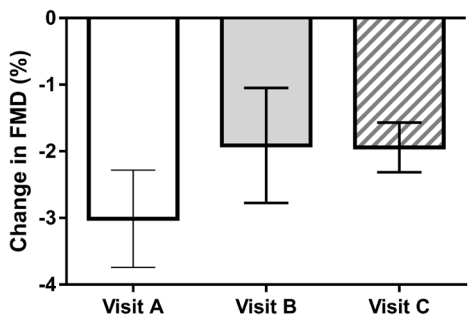

Figure 14. Absolute change in FMD between baseline and after ischemia-reperfusion at each visit in Study IV. Bars mark mean and whiskers mark SEM. p=0.39 for comparisons between visits. FMD = flow-mediated dilatation. SEM = standard error of the mean.

Table XIV. Brachial artery diameter, peak flow and endothelium-independent vasodilatation of the brachial artery from visits A-C in Study IV. Means and SEM. p>0.05 for comparisons within each variable. n=17. EIVD = endothelium-independent vasodilatation of the brachial artery; IR = ischemia-reperfusion.

	Visit A		**Visit B**		**Visit C**	
Variables	Before IR	After IR	Before IR	After IR	Before IR	After IR
Baseline diameter (mm)	3.8 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.2 ± 0.1
Vpeak (cm/s)	48 ± 3	43 ± 3	43 ± 2	43 ± 3	47 ± 3	43 ± 3
EIVD (%)	14.3 ± 1.5		13.3 ± 1.7		12.1 ± 1.4	

Table XV. Platelet inhibition in Study IV. Means and SEM. Significant difference from visit A within each test is depicted. ****p<0.0001. *p<0.05. n=17.

Variable	Visit A	Visit B	Visit C
Trc-ADP	23 ± 1.9	53 ± 3.9****	53 ± 4.6****
Trc-ASPI	15 ± 1.7	23 ± 1.9*	22 ± 2.0*

DISCUSSION

The concept of reperfusion injury has been extensively investigated since some 40 years. Although the precise underlying mechanisms are not fully understood, the therapeutic potential of therapies aimed at limiting reperfusion injury in STEMI is widely recognized. However, despite great scientific efforts and success in preclinical and early human studies, translation to clinical use has been cumbersome. Larger human trials exploring several different treatment strategies have not been conclusive and a therapy targeting reperfusion injury is yet to reach clinical guidelines. Nonetheless, RIC has emerged as a promising new approach, following the first trial of its effect in STEMI, demonstrating that RIC of the arm initiated during ambulance transport to the catheterization laboratory for primary PCI increased MSI [38]. In the light of that landmark study, the present thesis was undertaken to investigate if a clinically feasible RIC-protocol initiated upon arrival in the catheterization laboratory provides cardioprotection in patients with STEMI treated with primary PCI and furthermore to explore underlying mechanisms of RIC. In order to bring additional insights into the signaling mechanisms behind RIC, the involvement of the GLP-1 receptor in the protective effect of RIC against ischemia-reperfusion injury in humans was determined. Finally, the potential influence of co-medication with ticagrelor on ischemia-reperfusion injury was investigated in patients with previous ACS. In a randomized trial of patients with anterior STEMI, we found that RIC as an adjunct to primary PCI does not limit infarct size or improve short-term or long-term clinical outcomes. In a mechanistic study of healthy male subjects we discovered the previously unknown mode of action of RIC to induce protection against endothelial ischemia-reperfusion injury in humans through a GLP-1 receptor dependent pathway. Furthermore, we found that chronic treatment with ticagrelor does not protect against endothelial ischemia-reperfusion injury or improve basal endothelial function in patients with a previous ACS.

Effect of RIC in STEMI

For the randomized trial of patients with STEMI we chose to study only patients with signs and symptoms of anterior STEMI, aiming to include patients with large MaR, thereby optimizing the power of the study. This strategy was supported by previous trials that have indicated that the effect of cardioprotective interventions aimed at limiting ischemia-reperfusion injury may be most prominent in patients with large MaR[38,158,161]. We chose MSI as the primary endpoint as it evaluates infarct size relative to MaR, thereby limiting the impact of several factors (e.g. localization of coronary occlusion, temporal variation of degree of coronary occlusion and extent of collaterals) with the potential to affect the extent of ischemia and thereby absolute infarct size[162]. The influence of these factors increase the variability in infarct size of the study group resulting in the need of large sample sizes to detect significant differences in absolute infarct size. Therefore, using MSI as primary endpoint in our study with limited sample size was preferable. CMR was chosen as the modality to evaluate MSI as it allows to evaluate both MaR and infarct size in a single examination within a week from the myocardial infarction[150]. MaR was determined with early contrast-enhanced steady state free precession as it is a validated robust method, less sensitive to artifacts than T2-weighted analysis and has been used in previous multicenter trials and validated against SPECT[107,150,163].

Although achieving a major proportion of patients with large MaR, relatively short symptom time (ensuring that a substantial part of the MaR was salvageable), good quality CMR with low intra-observer variation and low drop-out of study subjects, we found no effect of RIC on infarct size or clinical outcomes. Relative (MSI) and absolute (myocardial scar, troponin T release and left ventricular function) markers of infarct size were neutral between study groups in acute and long-term comparisons. Likewise, cardiac mortality, MACCE and all its individual components were not altered up to three years.

While our findings may appear inconsistent with those reported from previous studies of RIC in STEMI[38-42,44-48,89,91], the different methodological designs and study endpoints used in the different trials complicate comparisons **(Table I och Table II)**. Several studies have demonstrated an effect on surrogate markers of myocardial injury such as enzyme levels or ST-segment resolution[39-42,44-47,91]. However, the significance of such surrogate markers in the RIC-setting is unknown. Only the studies by White et al. and Liu et al. confirmed their findings of reduced enzymatic levels with a more robust marker of absolute infarct size, using CMR[41,45]. However, relying on measures of absolute infarct size may be troublesome in studies of limited sample size as several factors may influence infarct size independently of the intervention studied[164-166]. While some of those factors may be measured and adjusted for, other factors such as temporal variation of degree of coronary occlusion and extent of collaterals are more difficult to reliably detect. The resulting variability in infarct size affects the statistical power of the trial and may obscure the findings. Evaluating infarct size relative to MaR, to some extent overcomes these issues and increases statistical power[167]. Infarct size (CMR LGE images) analyzed relative to the extent of myocardial edema was not different between study groups in the trials of Crimi et al. and White et al., using T2-weighted and T2-mapping techniques respectively for depiction of myocardial edema[40,41]. Interestingly, both the trial by Crimi et al. and the trial by White et al. found a smaller extent of myocardial edema on CMR in the groups treated with RIC than in the control groups, which led to the perception that the RIC intervention itself had reduced myocardial edema[40,41]. However, none of the trials were pre-specified for this endpoint. Nonetheless, a subsequent study in pigs found that RIC attenuated the extent of myocardial edema as measured by T2-weighted CMR[168]. Evaluation of MaR through T2-weighted and T2-mapping as well as early contrast-enhanced steady state free precession CMR sequences are edema-based and it has therefore been suggested that intervention with RIC may reduce the extent of edema and thereby MaR determined with CMR[169,170]. Intriguingly, using early contrast-enhanced steady state free precession CMR we noticed a larger MaR in the group treated with RIC. This was found to be associated to a larger proportion of proximal LAD occlusions in this group (50% more common), demonstrating the complexity in interpreting findings in this multifaceted research field. Furthermore, myocardial edema determined by CMR has also been argued to correlate better with the extent of myocardial infarction than with MaR[171]. Although these methods are validated and widely used to evaluate MaR, the above mentioned issues have questioned the appropriateness of their use to measure MaR in studies of cardioprotection[105,110,169,170].

While the basic underlying idea of the effect of RIC against ischemia-reperfusion injury in STEMI is a reduction of the final myocardial infarct size, the use of different markers of absolute or relative infarct size as outcome measures is motivated from a pathophysiological viewpoint. Theoretically, this enables detection of relatively small treatment effects and

allows for smaller sample sizes than in studies based on clinical endpoints. However, it is important to remember that such endpoints in clinical trials are highly sensitive to the impact of several factors (e.g. localization of coronary occlusion, temporal variation of degree of coronary occlusion and extent of collaterals), which may affect the results in studies of limited sample size. Albeit more resource-intensive, using clinical endpoints of mortality and morbidity is preferable to properly determine the clinical value of RIC.

The trial by Gaspar et al. is the only one so far having clinical outcomes as the primary endpoint[48]. However, some inconsistency in the results challenge the interpretation of the trial. While having no impact on troponin I levels, RIC was associated with a reduction in combined two-year cardiac mortality and hospitalization for heart failure as well as for the individual components. However, there was no effect on two-year all-cause mortality or MACCE (defined as death, myocardial infarction, stroke, or target vessel revascularization). Furthermore, left ventricular function at one year was not affected, but an association between RIC and improvement of left ventricular function at one-year follow-up was observed in the subgroup of patients with the initially most severely impaired left ventricular function. Of potential importance is that the higher proportion of triple vessel coronary artery disease in the control group may have affected outcomes. Eitel at al. found no effect on clinical outcomes at six months follow-up in patients treated with combined RIC and local postconditioning[43]. However, Stiermaier et al. recently reported long-term data from the same trial, showing a reduced incidence of major adverse cardiac events (cardiac death, re-infarction and new congestive HF) after a median of 3.6 years follow-up[90]. This result was driven by a lower rate of HF. Clinical outcomes were also reported from the trial by Crimi et al., showing no difference between groups regarding death, stroke, myocardial infarction, repeated PCI or coronary artery bypass grafting at one year follow-up[40]. Likewise, MACCE (hospital admissions due to ACS, heart failure, cardiac rupture, cardiac death or stroke) at 30 days were neutral between study groups in the trial by Yamanaka et al.[44], as were major adverse cardiac events (all-cause mortality, nonfatal myocardial infarction, and target vessel revascularization) at six months in the trial by Elbadawi at al.[46].

The most robust data supporting a cardioprotective effect of RIC in STEMI comes from the study by Bøtker et al.[38]. In spite of neutral results regarding troponin T levels, ST-resolution and myocardial scar, the primary outcome SPECT-based MSI was significantly larger in the group treated with RIC. A better left ventricular function within 24 hours of the intervention was noted. However, this difference did not persist after 30 days, at which time the incidence of major adverse coronary events likewise was equal between groups. Subgroup analysis showed that the effect of RIC on MSI was most evident in patients with large MaR and that this translated into better left ventricular function acutely, which persisted at 30 days[161]. A follow-up study (median time 3.8 years), found that MACCE (composite of all-cause mortality, myocardial infarction, readmission for heart failure and ischemic stroke/transient ischemic attack) was less prevalent in the RIC group[89].

Comparing our study with that of Bøtker et al. it is important to keep in mind some key differences. Our study was the first to use early contrast-enhanced steady state free precession CMR in a trial of RIC in STEMI. However, this method has been validated against SPECT, used in the trial by Bøtker et al. Importantly, we performed the RIC-stimulus during late ischemia and early reperfusion (per-post remote ischemic conditioning) and used a higher number of conditioning cycles. While underlying mechanisms involved in mediating

cardioprotection may differ when applying the RIC stimulus during late ischemia or early reperfusion[57], experimental trials have confirmed the efficacy of a per-post remote ischemic conditioning protocol[57, 124]. However, it is possible that it is too late to perform part of the conditioning protocol after the onset of reperfusion in the clinical setting of STEMI. Additionally, it has been suggested that too many RIC cycles may result in deleterious hyperconditioning that may erase the benefits of RIC[172]. However, relevant experimental evidence supporting this concern is lacking and exploratory subgroup analysis in our study based on the number of RIC cycles performed showed no trend towards an effect of RIC in patients receiving fewer conditioning cycles. Furthermore, the trial of Bøtker et al. and ours were undertaken during a slightly different time period with inherent differences in baseline STEMI treatment, which may have affected the outcomes. Of special interest is that we used predominantly ticagrelor in addition to aspirin to achieve dual antiplatelet therapy, whereas the trial by Bøtker et al. used clopidogrel. In this aspect it is important to consider the pleiotropic actions of ticagrelor beyond platelet inhibition to inhibit cell reuptake of adenosine[137] and the cardioprotective properties of adenosine[139, 140]. Thus, it is possible that patients were already receiving ticagrelor-induced cardioprotection, thereby masking a cardioprotective effect from RIC. Considering the proposed involvement of adenosine in mediating cardioprotection induced by RIC[59], one may in fact speculate that the cardioprotective route of RIC was already being utilized in both study groups of our trial by the means of increased adenosine action induced by ticagrelor, thereby blunting the effect of RIC. However, the uptake and effect of an oral loading dose of ticagrelor in patients with STEMI may be delayed, especially when simultaneously treated with morphine, further complicating interpretation[173]. Additionally, morphine itself is associated with cardioprotective properties[39], illustrating the intricacy of this field of research.

Collectively, data on the effect of RIC in STEMI remain inconclusive. While our study did not present support for its cardioprotective efficacy, the well-conducted landmark trial by Bøtker et al. argues the opposite. Considering the well acknowledged challenges of clinical studies in this field, further large scale clinical trials with appropriate endpoints are still warranted. Much awaited is the ongoing CONDI2/ERIC-PPCI (NCT02342522) multicenter trial randomizing more than 5000 patients with STEMI to a protocol of four cycles of upper arm RIC or sham procedure prior to primary PCI with cardiac death and hospitalization for heart failure as the primary outcome measure.

The GLP-1 receptor in RIC

While the cardioprotective potential of RIC in the ischemia-reperfusion setting is evident from numerous animal studies, the overall results from clinical trials so far have not been fully as conclusive. This has stressed the importance of decoding the mode of action of RIC. The startling transfer of the conditioning signal from remote tissue to end-organ has been intensely explored and several possible pathways have been proposed. Sectioning of the spinal cord, the vagal, the sciatic or the femoral nerves[50,55-57] abolishes cardioprotection provided by RIC, indicating the involvement of a neural route of transfer of the conditioning signal. This is further supported by the findings that the effect of RIC is inhibited by the muscarinic cholinoreceptor blocker atropine[50] and the autonomic ganglion blockers hexamethonium[35] and trimetaphan[37]. However, there is also strong evidence for the existence of a humoral route based on the finding that the isolated Langendorff-perfused heart is

protected by dialysate from a donor treated with RIC[61]. Remarkably, this is valid even across species[64,174,175]. Intriguingly, the effect is lost if the donor has undergone bilateral vagotomy[53], if the dialysate comes from a patient with diabetic neuropathy[49] or if the recipient heart is administered atropine or hexamethonium[53]. Collectively, these findings indicate an interplay between humoral and neural mechanisms to elicit end-organ protection from the remote stimulus. However, the site and mode of such interaction has not been identified.

In a recent study in rats it was demonstrated that selective sectioning of the posterior gastric branch of the vagal nerve abolishes cardioprotection from RIC, while stimulation of the same branch mimics the protection induced by RIC[69]. Subsequently, the hypothesis was raised that activation of the posterior gastric branch of the vagal nerve leads to release of a cardioprotective humoral factor from visceral organs receiving innervation from this branch. While several gut hormones could be potential candidates, most attention was directed towards the incretin peptide hormone GLP-1. GLP-1 has been increasingly appreciated for its cardioprotective virtues in patients with diabetes mellitus[176,177]. Importantly, the use of GLP-1 receptor agonists has appeared as one of the currently most promising approaches to pharmacologically limit ischemia-reperfusion injury after two trials demonstrating smaller infarct size and better left ventricular function in patients with STEMI by the administration of the GLP-1 receptor agonist exendin-4 as an adjunct to standard therapy including primary PCI[75,76]. Somewhat puzzling, a similar approach did not result in smaller infact size in another recent study[178]. Nontheless, in a rat model it was demonstrated that the GLP-1 receptor inhibitor exendin(9-39) abolishes RIC-induced cardioprotection[179]. Plasma GLP-1 levels were moderately increased in response to RIC. Furthermore, it was shown that systemic administration of the GLP-1 receptor agonist exendin-4 mimics the cardioprotective effect of RIC through an M3 muscarinic receptor-dependent mechanism which is insensitive to bilateral cervical vagotomy.

Based on these findings, Study III was conducted. The impact of GLP-1 receptor inhibition on the protective effect of RIC against endothelial ischemia-reperfusion injury was evaluated in healthy males to avoid the possible confounding effect of comorbidities, co-medication and menstrual cycle[180,181]. Albeit limiting the generalizability of the results, this study design was chosen as it served the purpose of exploring the signaling mechanisms behind RIC. Endothelial ischemia-reperfusion injury as evaluated by FMD was used as effect measure as it is a validated model for such investigations[123]. We could in this study confirm that ischemia-reperfusion is associated with a marked impairment of endothelial function. The study clearly demonstrated that intravenous infusion of the GLP-1 receptor antagonist exendin(9-39) abolishes the protective effect of RIC against endothelial ischemia-reperfusion injury. While subsequent studies need to confirm that this mechanism is present also in myocardial ischemia-reperfusion injury in humans, our findings propose a possible link between the neural and humoral mechanisms involved in mediation of the conditioning signal elicited by RIC. Study III was not designed to identify the specific mode of such an interaction. Nonetheless, combining our results with those of previous studies may suggest that RIC induces vagal activation, leading to the release of GLP-1 from intestinal L-cells to further relay the conditioning signal. However, the precise site of action of GLP-1 is still unclear. GLP-1 receptors are widely present in the body and abundant research has demonstrated their involvement in complex signaling pathways including the central neural system and various peripheral organs[182]. It has been shown that GLP-1 improves endothelial function via a GLP-1 receptor dependent mechanism[183-185], indicating that RIC may induce end-organ protection through the effect of systemic GLP-1 acting on the endothelium. However, forearm blood

flow is not increased by intra-arterial infusion of GLP-1 in healthy subjects[186]. Although not statistically significant, the small increase (20%) in plasma GLP-1 in Study III was comparable to that in rats experiencing GLP-1 receptor dependent cardioprotection from RIC[179]. The subtle change in plasma GLP-1 level in response to RIC in Study III makes it rather unlikely that the effect on the endothelium in this trial comes from circulating GLP-1. Another conceivable mechanism is that GLP-1 released from intestinal L-cells or at another, yet unidentified, site in the periphery stimulates GLP-1 receptors on vagal afferents, propagating the conditioning signal to the central nervous system, where it is further relayed towards end-organ protection. Interestingly vagal afferent fibers carrying GLP-1 receptors are involved in such neuroincretin circuits to control blood glucose levels and food intake[187]. Furthermore, the possible involvement of GLP-1 released locally in the central nervous system (perhaps as part of a vagal loop) to further communicate the conditioning signal needs to be taken in to account. Apart from being an incretin, GLP-1 is also an important neurotransmitter, released primarily in the solitary nucleus of the brainstem[188]. While predominantly explored for its metabolic effects, intracerebroventricular infusion of exendin-4 also increases heart rate and blood pressure through a pathway dependent on the vagal nerve, demonstrating the potential significance for cardiovascular effects[189]. Additionally, in a mouse model it was recently shown that the GLP-1 receptor agonist exendin-4 controls cardiac ventricular excitability in Langendorff-perfused hearts in an Ach and NO mediated manner, indicating the presence of GLP-1 receptors on efferent vagal neurons at the intrinsic cardiac ganglia[190]. Thus, it is conceivable that GLP-1 may in fact exert cardioprotection through dual pathways, involving receptors in the periphery as well as in the central nervous system and that RIC may activate one or both of those pathways. Interestingly, an additive cardioprotective effect of exendin-4 to RIC has been demonstrated in pigs[191]. These findings are indeed being further explored in the ongoing very interesting COMBAT-MI trial (NCT02404376) randomizing 428 patients with STEMI undergoing primary PCI to placebo, RIC, the GLP-1 receptor agonist exenatide or the combination of RIC and exenatide. In fact, the concept of providing cardioprotection through multitargeted approaches is currently gaining increased attention[192].

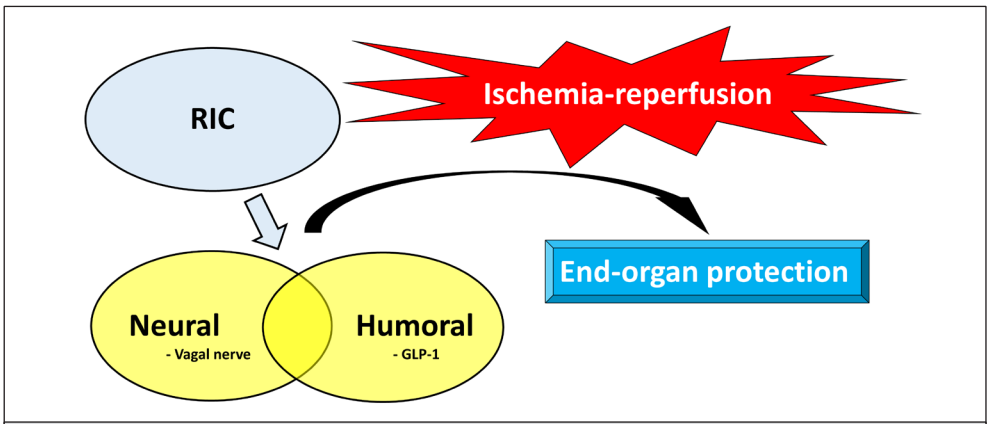

Figure 15. Proposed neurohormonal link for transmission of cardioprotective signal in RIC. RIC performed during ischemia-reperfusion initiates a cardioprotective interplay involving activation of the vagal nerve and the GLP-1 receptor and ultimately terminating in end-organ protection. GLP-1 = glucagon-like peptide-1; RIC = remote ischemic conditioning.

To summarize, the results of Study III demonstrate that RIC exerts end-organ protection through a GLP-1 receptor-dependent mechanism and, combined with data from previous studies, propose the vagal nerve as a link between humoral and neural pathways involved in mediating the effects of RIC **(Figure 15)**. However, the precise underlying structure of this mechanism is unclear and requires further exploration.

Effect of ticagrelor treatment on the endothelium

The effect of chronic ticagrelor treatment was evaluated in a clinically relevant cohort of patients with a previous ACS, thereby strengthening the generalizability of the study. Analyzing the degree of platelet inhibition with Multiplate (Roche Diagnostics) allowed us to confirm patient adherence to treatment and discontinuation. Using a validated model based on FMD[123], we found that in patients with a previous ACS, long-term treatment with ticagrelor is not associated with protection against ischemia-reperfusion injury or better endothelial function compared to after its discontinuation.

Over the last decade there has been increasing interest for the pleiotropic effects of ticagrelor, possibly contributing to cardioprotection. In the PLATO-trial, ticagrelor was associated with better cardiovascular outcomes and survival compared to clopidogrel[134]. The precise underlying mechanism for this superiority of ticagrelor has not been confirmed, but studies in rats[141,142], pigs[143] and dogs[193] have shown that despite a similar degree of platelet inhibition, treatment with ticagrelor reduces infarct size after myocardial ischemia-reperfusion to a greater extent than treatment with clopidogrel, indicating a pleiotropic cardioprotective effect. Apart from being a P2Y12 receptor blocker, ticagrelor also increases extracellular adenosine levels through inhibition of the equilibrative nucleoside transporter 1 (ENT1)[137], leading to impeded uptake of adenosine in erythrocytes. When exposed to ischemia-reperfusion, ENT1-null mice are cardioprotected in an adenosine-dependent manner compared to wild type animals, indicating a therapeutic potential of this pathway[194].

Adenosine itself has received much attention for its possible cardioprotective properties. Although trials of adenosine as an adjunct to reperfusion therapy in STEMI have provided conflicting evidence of its efficacy, the comparison of these studies has been complicated by their differing designs (e.g. mode of adenosine administration and dose, mode of reperfusion, ischemic time)[195]. Much encouraging, a large meta-analysis found evidence for a cardioprotective effect of intracoronary adenosine in addition to primary PCI in STEMI[140]. However, a recent randomized trial of intracoronary adenosine in addition to primary PCI in STEMI, showed not only lack of cardioprotection, but also a higher rate of adverse clinical outcomes in the adenosine treated group, further complicating the interpretation of the possible effect of adenosine in STEMI[196]. Collectively these findings highlight the need to better understand underlying mechanisms of adenosine in ischemia-reperfusion to tailor future clinical studies adequately to unravel the potential role of adenosine in STEMI. This may also be of importance to fully understand the cardioprotective properties of ticagrelor, in the light of previous studies indicating an important involvement of adenosine.

A loading dose of ticagrelor in patients with ACS induced a significant increase in plasma adenosine levels compared to a loading dose of clopidogrel and this effect was related to inhibition of adenosine uptake by erythrocytes[138]. In healthy subjects a loading dose of ticagrelor was shown to induce adenosine-mediated increase in coronary blood flow[197].

Pigs receiving a loading dose of ticagrelor before myocardial ischemia-reperfusion suffered a significantly smaller myocardial infarction than if loaded with clopidogrel, despite equal degree of platelet inhibition and this effect was adenosine receptor dependent[143]. Furthermore, chronic (7 days) ticagrelor treatment before myocardial ischemia-reperfusion in rats dose-dependently reduced infarct size in an adenosine receptor-dependent manner including downstream activation of eNOS and cyclooxygenase-2[141]. Clopidogrel treatment (7 days) had no such effect despite similar degree of platelet inhibition. Studies of patients with longer duration of ticagrelor treatment have yielded conflicting results. Patients with a previous ACS receiving ticagrelor for three to twelve months had better endothelial function than a control group on chronic clopidogrel or prasugrel treatment[198]. Ticagrelor treatment for 30 days improved endothelial function compared to clopidogrel in patients with a previous ACS and this was correlated to a significant increase in plasma adenosine levels[199]. In contrast, four weeks of treatment with ticagrelor in patients with a previous ACS was not associated with improvement in endothelial function compared to treatment with clopidogrel or prasugrel[200]. Additionally, plasma adenosine levels were unaffected. Similarly, termination of ticagrelor treatment after one year in patients with a previous ACS did not result in deterioration of endothelial function[201]. However, this assessment was performed within five days after cessation of ticagrelor treatment. Furthermore, in patients with stable coronary disease, plasma adenosine levels were not affected after 30 days of ticagrelor treatment compared to 30 days of clopidogrel treatment[202]. To summarize, there is robust support for the superiority of ticagrelor over clopidogrel for clinical outcomes in patients with ACS, despite similar degree of platelet inhibition between the compounds, indicating a pleiotropic effect of ticagrelor of clinical significance. Available experimental data supporting the idea that ticagrelor exerts an adenosine receptor dependent protection against myocardial ischemia-reperfusion injury come from animal studies, while previous mechanistic trials in humans have only investigated basal endothelial function per se and results are not conclusive.

Studies I and II were the first to evaluate the effect of RIC in STEMI in patients receiving predominantly ticagrelor in addition to aspirin to achieve double antiplatelet therapy. Considering the neutral outcome of those studies as opposed to the findings of the trial of Bøtker et al.[38], (where patients mainly received clopidogrel in addition to aspirin) it is worth contemplating whether patients in Studies I and II were already receiving cardioprotection through pleiotropic effects of ticagrelor. Thereby, the impact of further cardioprotection by RIC may have been blunted. Interestingly, there is support for the involvement of adenosine in mediating cardioprotection induced by RIC[59]. Thus, it is conceivable that the cardioprotective route via remote ischemic conditioning was already activated in both study groups by the means of increased adenosine action induced by ticagrelor. These considerations led us to conduct Study IV.

Study IV was the first to investigate the effect of ticagrelor on an established model of ischemia-reperfusion injury in humans. The demonstrated lack of protection against ischemia-reperfusion injury contrasts the findings of previous animal studies and illustrates the well-known translational challenges associated with research in cardioprotection[203]. Furthermore, discontinuation of chronic ticagrelor treatment did not result in decline in endothelial function per se. While this supports the findings of one previous trial[201], our study examined the subjects with a longer interval after cessation of ticagrelor and longer intervals between examinations, ruling out any possible lingering effect of the medication

(additionally confirmed by measurements of platelet inhibition) or conditioning state from previous examinations. Study IV adds substantial knowledge to the growing understanding of the pleiotropic effects of ticagrelor and its possible implications. Importantly, it contributes to the understanding of two major current cardiological dilemmas:

What is the optimal duration of ticagrelor treatment after STEMI? Primarily founded on the results of one large randomized trial showing a modest risk-benefit gain of prolonged treatment[135], current ESC Guidelines for the duration of dual antiplatelet therapy give a weak recommendation (IIb) for considering extended treatment with ticagrelor beyond twelve months after an ACS to patients with high ischemic and low bleeding risk[133]. Underlying mechanisms for this prolonged protection are not clear, but pleiotropic properties of ticagrelor beyond platelet inhibition have been suggested[136]. Defining the clinical significance of the pleiotropic effects of ticagrelor could be helpful to tailor its use. The findings of Study IV do not give support for the notion that long term treatment with ticagrelor improves endothelial function or protects against ischemia-reperfusion injury in patients with a previous ACS.

Does co-medication with ticagrelor interfere with the effect of cardioprotective interventions? The impact of co-medication in the translational dilemma of cardiovascular research is receiving increasing attention[204]. The possible adenosine receptor dependent cardioprotective effect of ticagrelor has been suggested to conceal cardioprotective effects of other interventions, such as in our own previous study of remote ischemic conditioning as an adjunct to primary PCI in patients with STEMI. However, the findings of Study IV do not give support to the idea that long term treatment with ticagrelor affects ischemia-reperfusion injury in humans with established coronary artery disease.

Limitations

It was not possible to blind the RIC procedure to patients, interventionalists or FMD examinators. However, the analyses of FMD examinations and CMR images were performed by investigators blinded to randomization. We did not perform core lab analysis of CMR examinations. However, all CMR images were independently assessed by two investigators (one with level III CMR certificate) and intra-observer variability was low. Limb ischemia in the individual study subjects undergoing RIC was not verified. However, pilot investigations on healthy subjects confirmed loss of peripheral pulsations verified with Doppler with inflation of the lower limb cuff. The number of patients included in Studies I and II was based on a sample size calculation for the primary endpoint of Study I (MSI). Thus analyses of clinical outcomes must be cautious. There was an 18 % loss of follow-up of CMR examinations between Study I and Study II. However, loss to clinical follow-up was low. The generalizability of the findings in Studies III and IV is limited by the choice to include only men. Only the effect of chronic ticagrelor treatment and its discontinuation was investigated and no extrapolation to the effects of a bolus dose of ticagrelor in the acute STEMI setting can be done. Basal FMD and endothelial ischemia-reperfusion injury in ticagrelor-naïve patients were not evaluated. Plasma adenosine was not measured and we are therefore unable to correlate results to the ability of chronic ticagrelor treatment to affect plasma adenosine levels. Plasma levels of ticagrelor were not measured. However, analyses of ticagrelor-specific platelet inhibition confirmed compliance to ticagrelor and then discontinuation as intended.

Concluding remarks and future perspectives

Despite intense efforts over the last decades and striking efficacy in a large number of studies of myocardial ischemia-reperfusion injury in animals as well as in human surrogate models, clinical studies of patients with STEMI have still not gathered enough support to bring RIC into clinical practice. Several possible reasons for this merit discussion.

The evidence for the mere existence of ischemia-reperfusion injury are from in vitro and animal studies. While there is data supporting specific pathophysiological mechanisms contributing to reperfusion injury, it is difficult to distinguish myocardial ischemic from reperfusion injury in the individual subject. The firmest evidence for the existence of reperfusion injury come from the concept that interventions applied after the moment of reperfusion may alter the extent of cardiomyocyte death. Although this concept is widely accepted it is valid to note the possibility that the intervention may be targeting reversible cardiomyocyte injury related to the ischemic trauma irrespective of the reperfusion. Nonetheless, from a pragmatic point of view this difference may be of subordinate importance. An intervention with the potential to limit the extent of myocardial injury is worth exploring regardless of its specific pathophysiological target. While findings from animal studies strongly support the actuality of reversible myocardial ischemia-reperfusion injury, the collective experience from human studies has been more difficult to interpret. Experimental studies using therapeutic intervention in different models of ischemia-reperfusion injury (such as endothelial ischemia-reperfusion injury in Studies III and IV of this thesis) give support for the existence of reversible ischemia-reperfusion injury in humans. However, it is important to remember that these are surrogate models of myocardial ischemia-reperfusion injury. Results in studies of interventions against myocardial ischemia-reperfusion injury in humans have been less consistent. Considering the relatively large number of different approaches to limit ischemia-reperfusion injury that have proven highly efficient in animal studies but rather disappointingly have failed to show a clear effect in clinical human trials of myocardial ischemia-reperfusion injury, it seems appropriate to keep in mind that this may in fact indicate a misconception of pathophysiological target.

Additional factors may explain the translational challenges from animal and experimental human studies to human clinical trials. There are fundamental differences in the pathophysiological mechanisms investigated. In animal studies of myocardial ischemia, coronary occlusion is essentially a purely mechanical procedure, while patients with STEMI typically undergo a more complex multifactorial process including thrombotic activation, inflammation and distress. Additionally, unlike experimental animals, patients with STEMI are often elderly with concomitant diseases and various ongoing medications. These are major issues, when considering the translational dilemmas. Advanced age[205], diabetes mellitus[206], acute hyperglycemia[207] and hypercholesterolemia[208] have been demonstrated to impair cardioprotection from RIC. Additionally, co-medication may affect results in clinical trials of RIC[209]. Interpretation of experimental human studies using surrogate endpoints for myocardial ischemia-reperfusion injury such as endothelial ischemia-reperfusion injury are hampered by several factors. Subjects are typically examined in a very different setting to that of STEMI (thrombotic activation, inflammation, distress etc.). The relevance of using surrogate endpoints such as endothelial ischemia-reperfusion injury may be limited since it has not been possible to clearly validate them to human myocardial ischemia-reperfusion injury. It is unclear whether mechanisms protecting against non-myocardial ischemia-

reperfusion injury are relevant for cardioprotection. Furthermore, there is substantial variation in several important details in STEMI. The presence of pre-infarction angina, extent of collaterals, co-medication, variability of oxygen demand during ischemia, duration of ischemia and temporal variation of coronary occlusion until final reperfusion may all affect final infarct size. Some of these variables are possible to measure to some extent, while other are virtually impossible to detect and thus adjust for. Several of these factors may affect the effect of cardioprotective measures and thus have an impact on the results of clinical studies in humans. Finally, trials of RIC in patients with STEMI have so far mainly aimed to evaluate the effect on myocardial infarct size using different markers of myocardial injury. As the complexity of STEMI makes absolute infarct size highly susceptible to several important confounders, evaluating infarct size relative to MaR is to prefer. CMR has been validated against the golden standard SPECT and has the advantage of allowing evaluation of infarct size and MaR in a single examination within a week from the myocardial infarction. While it has gained increasing popularity also in studies of RIC in STEMI, its validity in this field of research has been questioned. CMR uses myocardial edema as a surrogate for MaR and it is not certain whether RIC itself affects myocardial edema or not.

To conclude, the effect of RIC in patients with STEMI remains to be confirmed. Future studies should evaluate infarct size based on robust markers of myocardial injury. Most appropriate methods are currently CMR and SPECT. Considering the complex heterogeneity of STEMI, evaluation of absolute infarct size is sensitive to the impact of several possibly confounding factors, thus requiring large sample sizes. Evaluating infarct size relative to MaR allows for smaller sample sizes, but controversies of validity (CMR) and availability (SPECT) may trouble such approaches. Development of a method with the capability to reliably discriminate myocardial injury due to reperfusion from that due to ischemia could dramatically change the preconditions for this field of research. The concept of multitargeted cardioprotective approaches may provide new possibilities to establish efficient therapies against ischemia-reperfusion injury.

While this thesis illustrates the translational challenges and complexity of research in the field of myocardial ischemia-reperfusion injury, it points to the need for deeper understanding of the underlying mechanisms of ischemia-reperfusion injury and RIC as well as for large scale clinical trials powered for hard clinical endpoints to fully determine the value of RIC in STEMI. Furthermore, a novel pathway involved in RIC in humans is presented, possibly linking the humoral and neural pathways together and providing the basis for further experimental studies to disclose the underlying mechanisms of RIC.

CONCLUSIONS

1. RIC as an adjunct to primary PCI in patients with STEMI does not confer cardioprotection.

2. RIC protects against endothelial ischemia-reperfusion injury through a GLP-1 receptor dependent pathway in humans.

3. Chronic treatment with ticagrelor does not confer protection against endothelial ischemia-reperfusion injury in patients with a previous ACS.

4. Chronic treatment with ticagrelor is not associated with better endothelial function than after its discontinuation in patients with a previous ACS.

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